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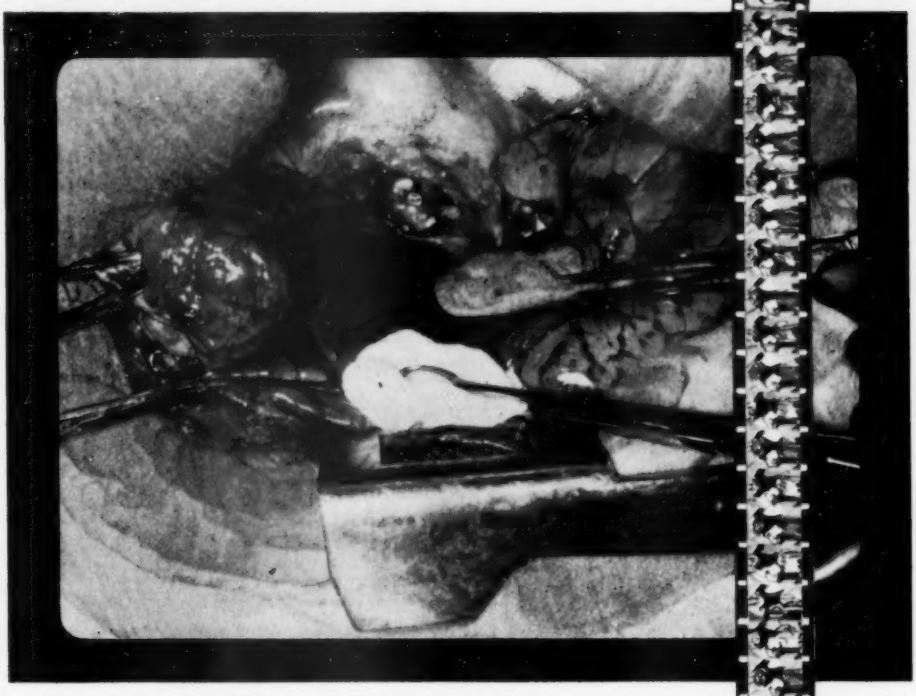

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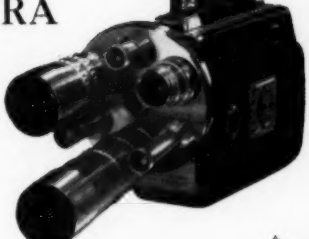

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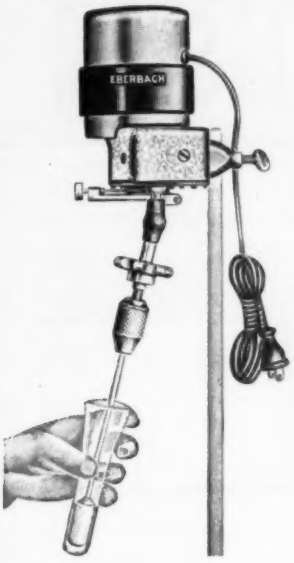

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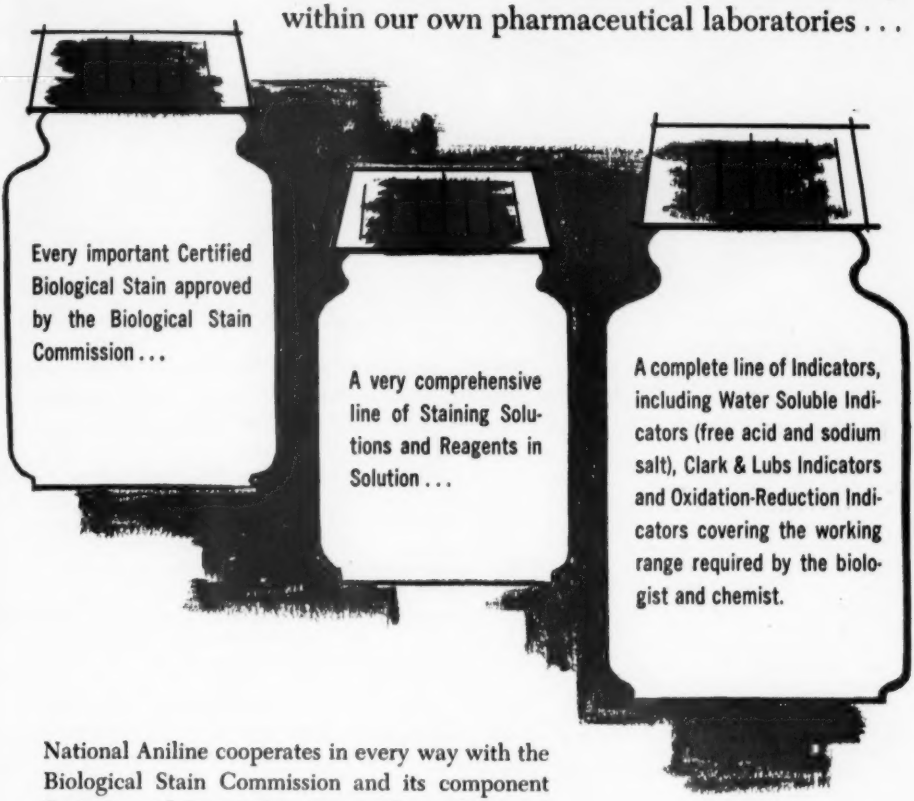
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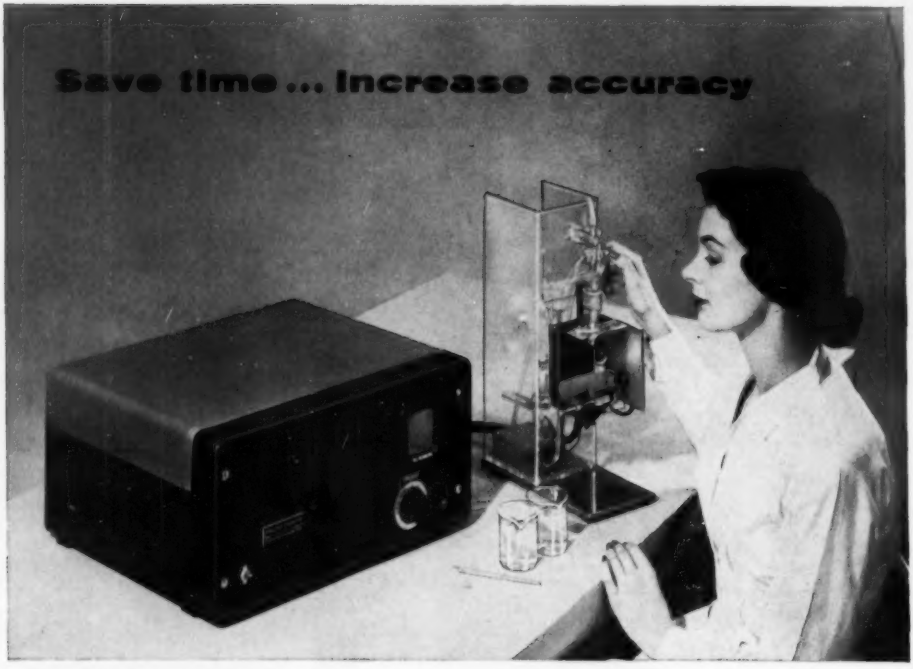

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Carcinogenic Studies on Water-Soluble and Insoluble Macromolecules

W. C. HUEPER, M.D., Bethesda, Md.

Numerous experimental investigations of the past decade have established the fact that various synthetic and semisynthetic, water-insoluble carbon and silicon polymers elicit sarcomas in rats and mice at the site of their subcutaneous or retroperitoneal implantation (Table 1).[1-23] Experimental studies of more recent years have shown that this property is shared by two water-soluble carbon polymers, namely, polyvinylpyrrolidone (PVP) and carboxymethylcellulose, when parenterally introduced into rats. Sarcomas developed at the site of their repeated subcutaneous injection in an aqueous solution, while reticulum-cell sarcomas of the liver and peritoneal lymph nodes represented the main neoplastic responses to the subcutaneous and intraperitoneal deposition of PVP in powder form or after its repeated intravenous introduction in saline solution into rats and mice.[24-26]

The target organs of carcinogenesis from water-soluble carbon polymers are under these conditions the tissues in which the macromolecular material is retained and stored and in which according to previous observations with these and other water-soluble synthetic, semisynthetic, and natural carbon polymers (polyvinyl alcohol, methylcellulose, pectin) a marked proliferation of reticuloendothelial cells and histiocytes accompanies the striking storage phenomena as well as the arterial atheromatosis developing after the parenteral administration of these colloids.[27-32] These proliferative and thesaurismotic reactions involving in part also the parenchymal cells of the liver, kidney, and brain and elicited by nonphysiologic macromolecules bear a close resemblance to those present in a number of spontaneous thesaurismoses which are characterized by the retention of lipoids, polysaccharides, mucopolysaccharides, and proteins of normal and abnormal types and of which some are associated with the occurrence of benign or malignant neoplastic lesions. Such physiologic thesaurismoses with neoplastic characteristics or complications are the various lipoidoses, such as essential xanthomatosis; Gaucher's disease; Niemann-Pick disease; the myocardial glycogenosis often accompanied by tumors of the brain, skin, and kidney; the mucopolysaccharidosis known as gargoylism; the microproteinosis associated with myelomatosis, and the megaloglobulinosis of Waldenström frequently associated with cancers of various organs but particularly those of the reticuloendothelial system. The experimental "polymer cancers" developing around the various synthetic and semisynthetic carbon and silicon polymers exhibit moreover a definite relationship to the pulmonary cancers induced in human lungs by the retention and in the vicinity of inhaled asbestos dust, which is a silicon oxide polymer.

Submitted for publication Oct. 31, 1958.
National Cancer Institute.

TABLE 1.—*Carcinogenic Macromolecular Carbon and Silicon Polymers*

Name	Description	Investigators
	I. Water-Insoluble Polymers	
	(*a*) Hydrocarbon Polymers Synthetic	
Polythene	Polyethylene	Oppenheimer et al., Druckrey et al., Bering & Handler
Lucite	Polymethylimethacrylate	Oppenheimer et al., Laskin et al., Zollinger
Polystyrol	Polyvinylbenzol	Oppenheimer et al.
Ivalon	Cross linked polyvinyl alcohol	Oppenheimer et al.
Dacron	Polyester condensate of terephthalate & ethylene glycol	Oppenheimer et al.
Bakelite	Phenol-formaldehyde condensate	Turner
	(*b*) Halogenated Hydrocarbon Polymers (Synthetic)	
Saran	Polyvinylene chloride	Oppenheimer et al.
PVC, Igelit, Vestolit, Vinnol	Polyvinyl chloride	Oppenheimer et al.
Teflon	Polyfluor (chlor)-olefine	Oppenheimer et al.
Pliofilm	Polymethylimethacrylate chloride	Oppenheimer et al.
Vinyon N, Dynel	Copolymer of vinyl chloride and acrylonitrile	Oppenheimer et al.
	(*c*) Aminized Hydrocarbon Polymers (Polyamides)	
Nylon	Polyhexamethylene diamine adipanide	Oppenheimer et al.
Perlon	Poly-e-caprolactam, polyurethane	Druckrey et al.
	(*d*) Hydrocarbon Polymers (Semisynthetic & Natural)	
Rubber	Processed latex gum	Druckrey et al., Mecke
Cellophane	Processed polyglucose (cellulose)	Oppenheimer et al., Druckrey et al., Körbler & Frank
Linen		
Parchment paper	Processed cellulose	Oppenheimer et al.
Silk		Oppenheimer et al.
Keratin		Oppenheimer et al.
Ivory		Nothdurft
	(*e*) Silicon Polymers	
Silastic	Polydimethylsiloxane processed (silicon rubber)	Oppenheimer et al., Mohr & Nothdurft
	II. Water-Soluble Polymers	
PVP	Polyvinylpyrrolidone	Hueper Lusky & Nelson
CMC, Collocel	Carboxymethylcellulose	Lusky & Nelson
Tween 60	Polyoxyethylene sorbitan monostearate	Lusky & Nelson

The discovery of polymer or macromolecular carcinogenesis has created important and urgent scientific as well as practical problems. A major controversy has developed concerning the type and the specificity of the causative mechanism active in the development of these polymer cancers. Some investigators (Druckery, Hueper) favored the view that specific chemical or physicochemical properties of these macromolecular agents are responsible for and active in the carcinogenic process. Such qualities may be related to specific endgroups, to macromolecular peroxide cross linkages, to metabolic fragments or molecular chemical abnormalities, to residual valencies, to trapped free radicals, to complex formation with protein or mucopolysaccharides or with their building stones or to their molecular size and shape, or to the presence of impurities, such as traces of the catalyst used in their production (Haddow, Fitzhugh). Other workers (Nothdurft, Oettel, Oppenheimer et al.) have concluded that the great chemical diversity of the polymers involved in carcinogenesis and the apparent dependence of this property upon the absolute size of the implants and upon the size of the unbroken surface of the implants of water-insoluble polymers and on their physical status—i. e., film, disk, fabric, thread or powder—militates against a specific chemical causal mechanism of polymer carcinogenesis. They have advanced the concept that the carcinogenesis of the polymerized substances depends upon their ability to elicit in the tissues the formation of a fibrous capsule from which in turn as the result of chronic nonspecific irritation and therefrom resulting continued cell proliferation sarcomas originate on a multicentric basis. The tendency toward the formation of a fibrohyaline capsule depends chiefly, according to these investigators, upon the physical status of the polymer, being most marked when polymers are implanted as films and being least developed when the polymer is present in powder form. Polymer carcinogenesis, according to this concept, is a nonspecific physical phenomenon, which is, in the opinion of its supporters (Nothdurft, Oppenheimer et al.), essentially analogous to the sarcogenesis obtained around disks of certain metals follow-

TABLE 2.—*Carcinogenic Metals and Mineral Foils and Powders*

Name	Description	Investigators
Mica	Metal silicates	Oppenheimer et al.
Glass ?	Metal silicate	Oppenheimer et al.
Quartz	Silicon oxide polymer	Druckrey et al.
Asbestos	Metal silicon oxide polymer	Druckrey et al.
Mercury		Druckrey et al.
Silver		Nothdurft
Gold		Nothdurft
Platinum		Nothdurft
Stainless steel	Alloy of nickel, chromium, iron	Oppenheimer et al.
Steel		Oppenheimer et al.
Tantalum		Oppenheimer et al.
Vitallium	Alloy of cobalt & chromium	Oppenheimer et al.
Beryllium		Barnes et al., Gardner; Dutra et al.
Nickel		Hueper
Chromium		Hueper, Schinz
Cobalt		Heath, Schinz
Uranium		Hueper

ing their implantation into the subcutaneous tissue of rats (Table 2).[33-39]

Apart from the distinct scientific importance which these two divergent concepts on polymer carcinogenesis possess, they are also of immediate practical significance, since they must influence the carcinogenic screening techniques applied to polymers, as well as the assessment of possible carcinogenic hazards connected with exposure to the same polymer when present in different physical states and when incorporated in or in contact with consumer goods or when introduced into experimental animals or man by various routes.

The second and practical problem of major importance resulting from these observations on polymer carcinogenesis is closely linked to their use in the human economy. Water-soluble and insoluble macromolecular polymers of various types are being introduced into the human environment to a rapidly increasing degree and are being employed in numerous consumer goods, such as medicinal and cosmetic preparations, medicinal protheses, food and medicinal containers, packagings and coatings, household and sanitary goods, paints, dinnerware, and clothing. The human population, therefore, has frequent and intimate contact with polymers. The observations made on experimental animals, therefore, raises the question whether or not carbon and silicon polymers coming in contact with human tissues by various routes and in different forms may have a carcinogenic effect also upon man. Because of the possibility of a potential carcinogenic hazard to man posed by previous studies on polymers, a greatly extended experimental study of carbon and silicon polymers for elucidating the chemical and physical properties underlying their carcinogenicity and their carcinogenic mechanism was urgently needed.

Experimental Procedure

The experiments to be reported were conducted for these reasons on various water-soluble and insoluble carbon and silicon polymers. Ten PVP's were tested. Of these, four were of technical grade and three were of medicinal type, while three others were specially prepared for this study by American and German manufacturers. These three PVP's had a rather low average molecular weight, ranging from 10,000 to 23,000. Two of them, moreover, had a narrow molecular weight range. The average molecular weights of the entire PVP series ranged from approximately 10,000 to about 300,000. Six of them were made in Germany; three, in the United States, and one, in Great Britain. Different catalysts were used in their production, depending upon the manufacturer.

In an additional experiment, a polyvinyl alcohol preparation of American make and with an average molecular weight of 120,000 was used.

A third series of experiments comprised the testing of various polyglucoses. Eleven different dextrans manufactured by an American pharmaceutical concern and by the Regional Laboratory in Peoria, Ill., were used. These dextrans not only varied in molecular weight which covered a range between 37,000 and several million, but they differed among each other also in regard to their molecular shape. Some consisted of linear molecules, while others showed varying degrees of branching and thus had an almost globular shape. Other polyglucoses tested were a brand of methylcellulose with an average molecular weight of 140,000, an ethylcellulose of rather low molecular weight, and a soluble starch.

In addition to ethylcellouse the water-insoluble polymers studied were as follows: 1. Silastic, which is a processed polymer of dimethlysiloxane. This is vulcanized by the addition of 2,4-

TABLE 3.—*Carcinogenic Tests on Macromolecular Substances—Water-Soluble Polymers*

Chemical	Type	Average Mol. W.	Route	Dose, Mg. Single	Dose, Mg. Total	Species	Animals, No.
PVP 1	Technical	20,000	Subcut.	200	200	C57bl. mouse	50
PVP 2		20,000	Powder				
PVP 3		50,000	Intraper. powder	200	200	C57bl. mouse	50
PVP 4		300,000	Subcut. powder	500	500	Beth. bl. rat	30
			Intraper. powder	500	500	Beth. bl. rat	30
			Intraven. 7% sol.	18.5	150	Beth. bl. rat	15
			Intraven. 7% sol.	2,500	22,000	Dutch rabbit	3-6
PVP 6	Medicinal	50,000	Intraper.	1,000	15,000	Beth. bl. rat	20
PVP 7		50,000	20% sol.				30
			Intraven.	3,500	56,000	Dutch rabbit	6
			7% sol.	3,500	56,000	Dutch rabbit	6
			Subcut. powder	200	200	Beth. bl. rat	30
			Subcut. Rep. powder	200	600	Beth. bl. rat	30
Plasmosan		50,000	3.5% sol.	1,700	12,600	Dutch rabbit	6
PVP 5	Special	10,000	Subcut.	200	200	C57bl. mouse	30
			Powder	500	500	Beth. bl. rat	20
			Intraper.	200	200	C57bl. mouse	30
			Powder	500	500	Beth. bl. rat	20
PVP K17		18,000	Intraper.	500	2,000	Beth. bl. rat	30
PVP K25		23,000	25% sol.	6,250	62,500	Dutch rabbit	6
Polyvinyl alcohol	Technical	120,000	Subcut. powder	500	500	Beth. bl. rat	25
Methylcellulose	Technical	140,000	Subcut. powder	500	500	Beth. bl. rat	25
Soluble starch	Technical		Subcut. 45% sol.	500	500	Wistar rat	30
			Intraper.	500	500	Wistar rat	30
Dextran 1	Linear	200,000	Subcut.	200	200	C57bl. mouse	40
Dextran 2	Linear	100,000	Powder	500	500	Beth. bl. rat	20
Dextran 8	Branched	37,500	Intraper.	200	200	C57bl. mouse	20
Dextran 9	Branched	300,000	Powder	500	500	Beth. bl. rat	20
Dextran 10	Branched	89,400	Intraven.	1,750	17,500 to 35,500	Dutch rabbit	2-4
Dextran 11	Highly br.	71,400	7% sol.				
Dextran 3		Sev. million	Subcut.	200	200	C57bl. mouse	35
Dextran 4	Linear	75,000	Subcut.	200	200	C57bl. mouse	30
Dextran 5	Highly br.		Subcut.	200	200	C57bl. mouse	28
Dextran 6	Highly br.		Subcut.	200	200	C57bl. mouse	13
Dextran 7	Highly br.		Subcut; powder	200	200	C57bl. mouse	9

dichlorbenzoyl peroxide. Through the action of this chemical on the latex the dimethylsiloxane polymer molecule is altered from a straight linear molecule into a net molecule because of the formation of numerous cross linkages between the macromolecular chain molecules. The average molecular weight of the thereby resulting silicone rubber ranges from 300,000 to 400,000. The rubber contains, moreover, as a filler and plasticizer, a specially prepared and very pure silicon dioxide, which in the silicon rubber takes the place of carbon black used in the manufacture of natural or synthetic carbon rubber. 2. Polyethylene. 3. Polyurethane (Mondur TD-80), which is a modified polymer of tolylene-diisocyanate.

Pertinent data relative to the various macromolecular substances used as well as information on the general scheme of experimental investigations applied are presented in Tables 3 and 4.

The various water-soluble macromolecules were investigated for carcinogenic properties by administering them in single or repeated doses either as powders or as aqueous solutions through subcutaneous, intraperitoneal, and intravenous routes so as to determine the possible role which differences in chemical composition as well as in molecular size and shape might play in bringing about these responses. The water-insoluble polymers, on the other hand, were studied for ascertaining whether differences in the physical state of these substances —i. e., whether their presence in the form of cubes, balls, sheets (disks), films, sponges, or powders— is important in determining the occurrence and degree of cancerous reactions to parenterally implanted water-insoluble polymers. Rabbits, rats, and mice were employed for obtaining information on the species-specific character and range of such manifestations.

TABLE 4.—*Carcinogenic Tests on Macromolecular Substances—Water-Insoluble Polymers*

Chemical	Type	Average Mol. W.	Route	Dose, Mg. Single	Dose, Mg. Total	Physical State	Species	Animals, No.
Ethylcellulose	Technical	Low viscosity	Subcut.	500	500	Powder	Beth. bl. rat	25
Polyethylene			Intraper.	65	65	Cube 3.5×3.5×3 mm.	Beth. bl. rat	30
				65	65	Sheet 0.5 mm. thick, disk 12 mm. diam.	Beth. bl. rat	30
				65	65	Film 10×17.5 mm.	Beth. bl. rat	30
				65	65	Powder	Beth. bl. rat	30
Polyurethane			Subcut.	65	65	Sheet 2mm. thick 2 disks 3mm, 5mm. diam.	Beth. bl. rat	30
				65	65	Sponge 25×20×3 mm.	Beth. bl. rat	30
				65	65	Powder	Beth. bl. rat	30
			Intraper.	65	65	Sheet	Beth. bl. rat	30
				65	65	Sponge	Beth. bl. rat	30
				65	65	Powder	Beth. bl. rat	30
Polydimethylsiloxane (latex) (gum)		High viscosity	Subcut.	300	300	Ball	Beth. bl. rat	30
			Intraper.	300	300	Ball, 5 mm. diam.	Beth. bl. rat	30
Polydimethylsiloxane rubber (Silastic)			Subcut.	300	300	Cube	Beth. bl. rat	30
			Intraper.	300	300	Cube 5 mm. diam.	Beth. bl. rat	30
Silica			Subcut.	300	300	Powder	Beth. bl. rat	30
			Intraper.	300	300	Powder	Beth. bl. rat	30

The aqueous solutions of the polymers used were prepared with isotonic saline and were autoclaved for insuring their sterility. PVP (Plasmosan), as well as the solutions of PVP K17 and PVP K25, was supplied in ampules. The maximal observation period for mice and rats was two years; for rabbits, up to four years. All survivors were killed at the end of this period. Autopsies were performed on all animals. Histologic examinations of the tissues were made on all animals exhibiting grossly demonstrable pathologic changes or on at least 30% of animals of each series regardless of the presence or absence of such lesions. The organs of all rabbits were studied histologically. Normal untreated animals of the same strain and animals given parenteral implants or injections of various noncarcinogenic vehicles (wool fat, gelatin, tricaprylin) or test chemicals served as controls. They were permitted to live for the same length of time as the animals of the experimental series and were used for determining the type, age, and site distribution of spontaneous tumors.

The neoplastic lesions observed among the animals of the different test series in relation to the species and polymers used and the routes of their administration employed are listed in Table 5.

Experimental Observations

In the assessment of the relative significance of the cancers observed in the various experimental series in relation to the different polymers administered, the following criteria were considered:

(*a*) The absolute and relative number of cancers in a specific test series.

(*b*) The number of animals in a particular test series.

(*c*) The degree of uniformity of neoplastic response in several test series in which the same polymer was given to the same species by different routes.

(*d*) The degree of uniformity of neoplastic responses in test series in which different species were employed and to which the same polymer was administered.

(*e*) The topographical distribution of cancers in specific organs and tissues and their histogenetic types in their relation to the sites of retention and/or storage of the polymer introduced, i. e., the identity of the site of the implanted or stored polymer with the site of the subsequently developing proliferative hyperplastic and precancerous lesions and cancers.

(*f*) The relative frequency of spontaneously occurring cancers of the same type and site in control series of normal untreat-

TABLE 5.—*Neoplastic Reactions in Mice, Rats, and Rabbits Following*

				Sites & Types										
				Lymph Nodes			Liver			Lung		Heart	Breast	
Chemical Molecular Weight	Route Admin.	Species	No. Animals	Lymphoma	Leukemia	Ret. Sarcoma	Cholangioma	Ret. Sarcoma	Leukemia	Adenoma	Mesothelioma	Mesothelioma	Adenofibroma	
PVP 1	Subcut.	Mouse	50											
	In. per.		50											
20,000	Subcut.	Rat	30			4		2						
	In. per.		30			4	1							
	In. ven.		15			1		1						
	In. ven.	Rabbit	6											
PVP 2	Subcut.	Mouse	50	3										
	In. per.		50					1						
20,000	Subcut.	Rat	30				2							
	In. per.		30			1							1	
	In. ven.		15										1	
	In. ven.	Rabbit	4											
PVP 3	Subcut.	Mouse	50											
	In. per.		50	3										
50,000	Subcut.	Rat	30			5		2	1			1		
	In. per.		30				1		1			1		
	In. ven.		15	3										
	In. ven.	Rabbit	3											
PVP 4	Subcut.	Mouse	50	1										
	In. per.		50											
300,000	Subcut	Rat	30			4		2				1		
	In per.		30			6							1	
	In. ven.		15			2								
	In. ven.	Rabbit	2											
PVP 5	Subcut.	Mouse	30											
10,000	In. per.		30	1								1		
	Subcut.	Rat	20			1		3						
	In. per.		20			2		1						
PVP 6	In. per.	Rat	20	Unfinished										
	Subcut.	Rat	30			4		1						
50,000	Rep. sbc.		30			1		1						
	In. ven.	Rabbit	6											
PVP 7	In. per.	Rat	30	Unfinished										
50,000	Subcut.	Rat	30					2						
	Rep. sbc.		30			2		1					2	
	In. ven.	Rabbit	6											
Plasmosan 50,000	In. ven.	Rabbit	6											
PVP K17	In. per.	Rat	35	Unfinished										
18,000	In. per.	Rabbit	6											
PVP K25	In. per.	Rat	35	Unfinished										
23,000	In. per.	Rabbit	6											
PVA 120,000	Subcut.	Rat	25				1	4			1		2	
Methocel 140,000	Subcut.	Rat	25											
Dextran 1	Subcut.	Mouse	40	1	1			2						
200,000	In. per.		20	1										
	Subcut.	Rat	20			2		1					2	
	In. per.		20			1							2	
	In. ven.	Rabbit	3											
Dextran 2	Subcut.	Mouse	40					1						
100,000	In. per.		20					4		2				
	Subcut.	Rat	20			1						1	1	
	In. per.		20				1	2						
	In. ven.	Rabbit	3											
Dextran 3 Sev. million	Subcut.	Mouse	35	1						1				
Dextran 4 75,000	Subcut.	Mouse	30			1								

of Tumors

Kidney	Skin		Uterus	Ovary		Adrenal		No. & % Tumors					
								Total		Benign		Malig.	
Carcinoma	Carcinoma	Sarcoma	Carcinoma	Cystadenoma	Carcinoma	Hemangioma	Adenoma	No.	%	No.	%	No.	%
								0					
								0					
						1		7	23	1	3	6	20
	1		2	2				10	33	3	10	7	23
			1					3	20			3	20
								0					
								3	6			3	6
								1	2			1	2
			1	1				4	13	3	10	1	3
			1	3				6	20	5	17	1	3
			1					2	13	2	12	1	1
								0					
								0					
								3	6			3	6
				1		1		11	37	2	7	9	30
			3	1				7	23	2	7	5	16
			2				1	6	40	1	7	5	33
								0					
								1	2			1	2
								0					
	1		1	2				11	37	2	7	9	30
			2					9	30	1	3	8	27
			1					3	20			3	20
								0					
								0					
								1	3			1	3
			2					6	30			6	30
								4	20			4	20
			2					7	23			7	23
			6					8	27			8	27
								0					
								2	7			2	7
			2					7	23	2	7	5	16
								0					
			1					9	36	3	12	6	24
			1					1	4			1	4
								4	10			4	10
								1	5			1	5
			2					7	35	2	10	7	35
			1					4	20	2	10	2	10
								0					
								1	2			1	2
								6	30	2	10	4	20
								3	15	1	5	2	10
			1					4	20	1	5	3	15
								0					
								2	6	1	3	1	3
								1	3			1	3

TABLE 5.—*Neoplastic Reactions in Mice, Rats, and Rabbits Following*

Chemical Molecular Weight	Route Admin.	Species	No. Animals	Sites & Types: Lymph Nodes: Lymphoma	Lymph Nodes: Leukemia	Liver: Ret. Sarcoma	Liver: Cholangioma	Liver: Ret. Sarcoma	Liver: Leukemia	Lung: Adenoma	Lung: Mesothelioma	Heart: Mesothelioma	Breast: Adenofibroma
Dextran 5 H. Br.	Subcut.	Mouse	28			3							
Dextran 6 H. Br.	Subcut.	Mouse	13										
Dextran 7 H. Br.	Subcut.	Mouse	9			1							
Dextran 8 37,000	Subcut.	Mouse	40	1									
	In. per.		20	1									
	Subcut.	Rat	20				1		1				1
	In. ven.	Rabbit	3										
Dextran 9 300,000	Subcut.	Mouse	40			1							
	In. per.		20	1	1								
	Subcut.	Rat	20										
	In. per.		20			2		2					
	In. ven.	Rabbit	3										
Dextran 10 89,400	Subcut.	Mouse	40	1				2					
	In. per.		20					4					
	Subcut.	Rat	20			1	1	3					1
	In. per.		20				1	2					
	In. ven.	Rabbit	3	1					1				
Dextran 11 71,400	Subcut.	Mouse	40					2	1				
	In. per.		20										
	Subcut.	Rat	20					1					
	In. per.		20			2							1
	In. ven.	Rabbit	3							2			
Starch	Subcut.	Rat	30										6
	In. per.		30			1							1
Eth. cel.	Subcut.	Rat	25							1			
Silastic rubber	Subcut.	Rat	30				2	1			2		1
	Int. per.		30									1	
Polysil. latex	Subcut.	Rat	30										1
	Int. per.		30			2					2		
Silica	Subcut.	Rat	45			1	1	2			2		
	Int. per.		30					2					1
Polyurethane	Subcut.; int. per. disk; sponge; powder		6×30	Unfinished									
Polyethylene	Int. per. cube; disk; film 4×30; powder			Unfinished									
Normal controls		Rat	200			7		4			1		3
Treated controls		Mouse	840			6							

ed animals or those treated with noncarcinogenic chemicals.

When evaluating the data contained in Table 4 by applying the above listed criteria, carcinogenic responses attributable to the action of the polymer parenterally introduced into mice, rats, and rabbits are present for the following polymers: PVP 1, PVP 3, PVP 4, PVP 5, PVP 6, Dextran 10, Silastic rubber, and PVA. Such a relationship is probable but not definite for PVP 2, PVP 7, Dextran 1, Dextran 2, Dextran 5, Dextran 11, Polysilicone latex, and silica. For all other polymers, although some of them belong to the same general chemical group as the ones adjudged to be carcinogenic, the

a Parenteral Introduction of Water-Soluble and Insoluble Polymers—Continued

of Tumors

Kidney	Skin		Uterus	Ovary		Adrenal		No. & % Tumors					
								Total		Benign		Malig.	
Carcinoma	Carcinoma	Sarcoma	Carcinoma	Cystadenoma	Carcinoma	Hemangioma	Adenoma	No.	%	No.	%	No.	%
								3	10			3	10
								0					
								1	10			1	10
								1	2			1	2
								1	4			1	4
			1					4	20	2	10	2	10
								0					
								1	2			1	2
								2	10			2	10
			2	1				3	15	1	5	2	10
			1					5	25			5	25
								0					
								3	8			3	8
								4	20			4	20
1			1					8	40	2	10	6	30
			1					4	20	1	7	3	13
								2	70	1	35	1	35
								3	7			3	7
								0					
			1					2	10			2	10
			1					4	20	1	7	3	13
								2	66	2	66		
								6	20	6	20		
					1		1	4	12	2	6	2	6
			2					3	12			3	12
		10						16	55	3	10	13	43
								1	3			1	3
		1						2	6	1	3	1	3
			1					5	17			5	17
			1	1				8	18	2	4	6	14
			3					6	20	1	3	5	17
1			4		1			20	10	3	1	17	9
								6	0.7			6	0.7

experimental observations failed to demonstrate carcinogenic properties.

Whenever the cancer rate in a test series was found to be two to three times that of the control series, and especially when such a ratio was present following the introduction of a particular polymer by several routes and into several species and whenever the cancers involved organs and tissues in which the particular polymer had elicited storage phenomena and hyperplastic and precancerous cellular responses related to these reactions, the macromolecular substance in question was considered as possessing carcinogenic properties. The evidence

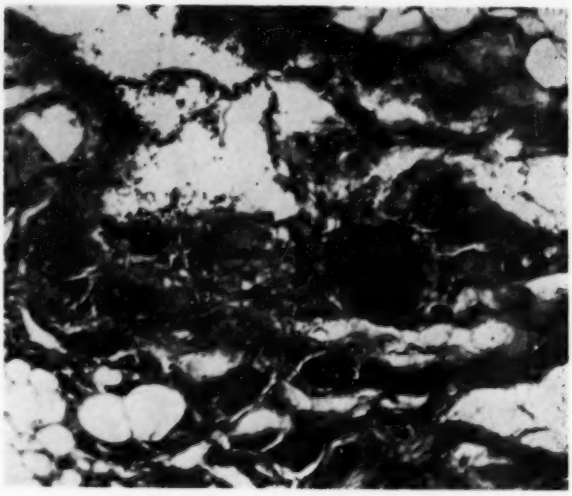


Fig. 1.—Fat tissue with giant cells filled with clumps of blue-stained matter (PVP) (rat).

underlying these conclusions is presented in the data listed in Table 5.

The histopathologic findings pertinent in this respect for the different polymers investigated are as follows:

1. Polyvinylpyrrolidones.—(*a*) Thesaurismotic Reactions: Storage reactions which were characterized by the appearance of bluish-stained material representing probably a PVP protein or mucopolysaccharide complex in usually swollen cells were observed in many organs and tissues of mice, rats, and rabbits, regardless of the molecular weight of the various PVP's tested. The degree of storage reactions depended mainly on the relative amounts of PVP administered and varied considerably between different series of animals and between animals belonging to the same series. In general they were most marked in rabbits, particularly those intravenously injected with Plasmosan and PVP's 6 and 7, i. e., the medicinal PVP's tested. They were somewhat less pronounced in rats and affected least the mice.

Fat tissue at the site of implantation contained often blue-stained phagocytic cells scattered throughout an otherwise normal tissue. Occasionally foci of huge multinu-

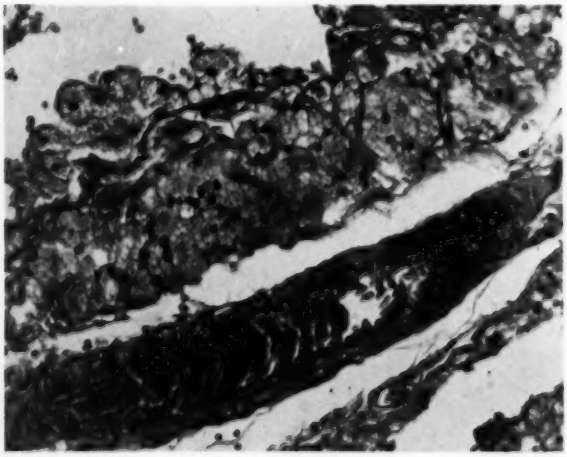


Fig. 2.—Meningeal membrane with foam cells with blue tinted cytoplasm (PVP) (rabbit).

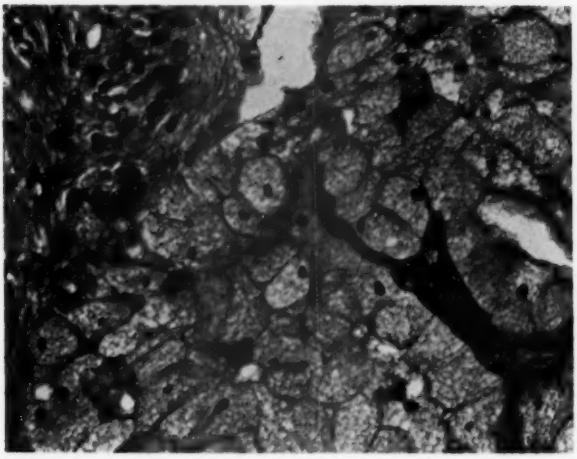


Fig. 3. — Choroid plexus with massive proliferation of foam cells with bluish stained cytoplasm; glia cells with blue cytoplasm in adjacent brain tissue (PVP) (rabbit).

cleated foam cells with bluish cytoplasm were found in the fat tissue (Fig. 1).

Massive accumulations of blue matter containing foam cells were noted in the meninges of rabbits given PVP 6 and 7 and Plasmosan (Fig. 2). In some animals this condition was associated with a distinct increase of histiocytic elements in the meninges. The ependymal cells as well as the cells of the choroid stroma were usually balloon-like, had a foamy and often blue-stained cytoplasm, and formed grape-like proliferations in the ventricles (Fig. 3). Groups of multinucleated giant cells were sometimes present in these masses (Fig. 4), while swollen and blue matter containing glia cells were noted at times in the adjacent brain tissue. In a few rabbits and mice also glia and ganglion cells of the basal ganglionic region exhibited a distinct blue stipling of their swollen cytoplasm. Cells with a bluish-stained cytoplasm were occasionally found also within peripheral nerves.

The lungs not infrequently revealed clusters of blue-stained foam cells located in the interstitial tissue. Giant-cell granulomas often were present in pulmonary capillaries occluding their lumens. The lumens of

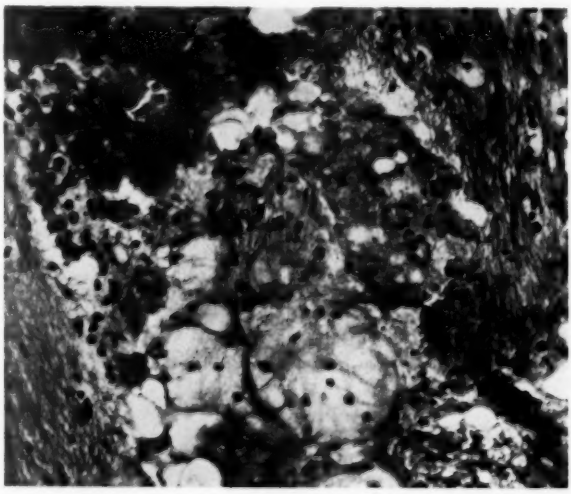


Fig. 4. — Choroid plexus with foam cells and giant cells with smudge-like clumps of blue-stained matter (PVP) (rabbit).

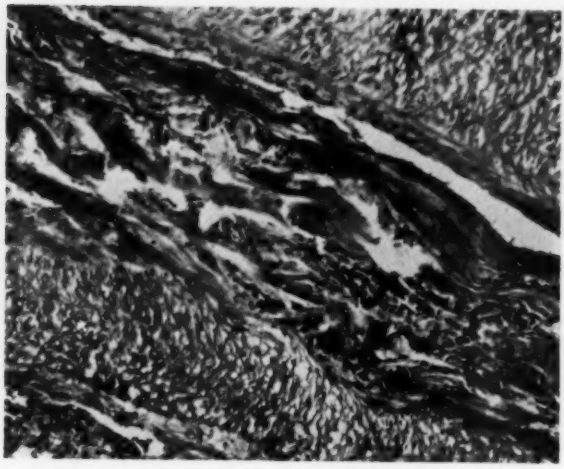


Fig. 5.—Pulmonary artery with an occluding foreign-body giant-cell granuloma (PVP) (rabbit).

medium-sized and large pulmonary arteries sometimes contained similar and often large giant-cell granulomas (Fig. 5).

Retention of PVP in the endothelial cells of the aortic intima of rabbits was not uncommon, producing a blue, bead-like cellular lining and being associated with an increase of endothelial cells. Such reactions sometimes resulted in the production of intimal foam-cell cushions in the ascending aorta, representing an experimental type of atherosclerosis (Fig. 6). Similar reactions were observed in the aortic leaflets as well as in the subendocardial tissue. Myocardial infiltrations with blue-stained foam cells were frequent and were usually most pronounced in the subepicardial area, where they gave rise to multiple focal accumulations of blue-stained foam cells.

Blue-stained matter was often noted in both liver cells and swollen Kupffer cells, which not infrequently were transformed into giant cells. Histiocytic balloon-like swollen blue foam cells were present in the periportal tissue, which occasionally was markedly increased in amount and which contained giant-cell granulomas (Fig. 7).

Mediastinal as well as abdominal lymph nodes revealed at times foci of blue-stained foam cells. Similar alterations occurred in

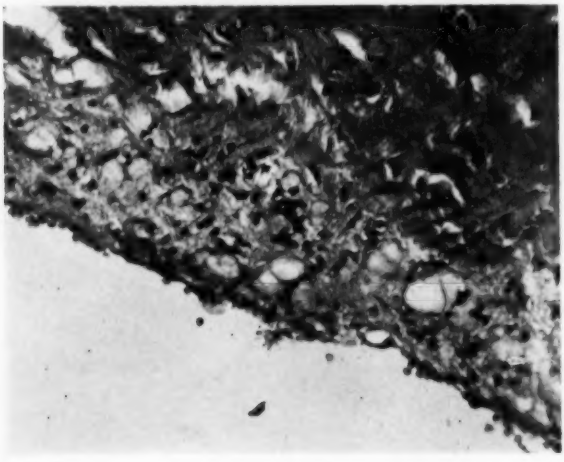


Fig. 6.—Ascending aorta with an intimal foam-cell cushion containing blue-stained matter (PVP) (rabbit).

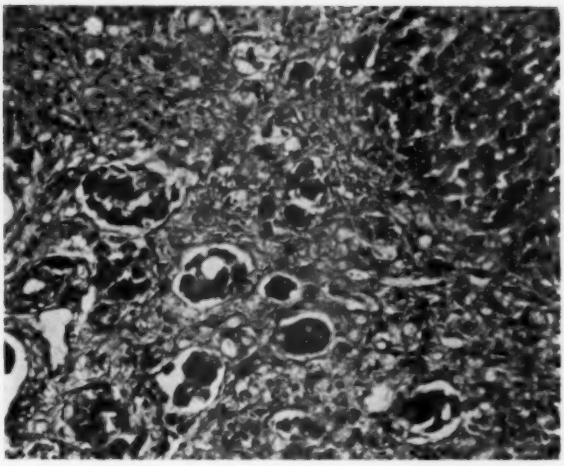


Fig. 7.—Liver of rat with interstitial fibrosis and clusters of foam cells and giant cells containing blue-colored material (PVP).

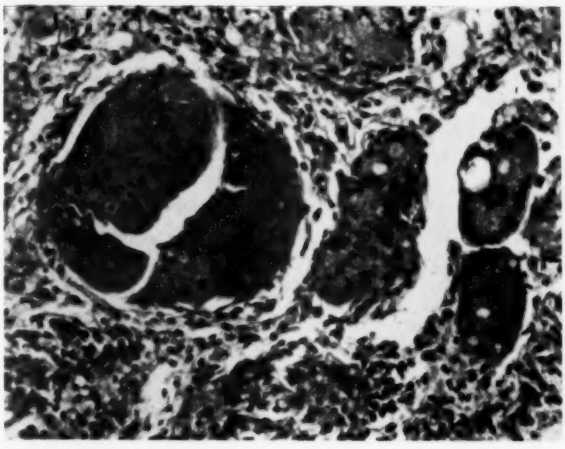


Fig. 8. — Clusters of multinucleated giant cells in the splenic pulp containing blue-stained foam cells (PVP) (rabbit).

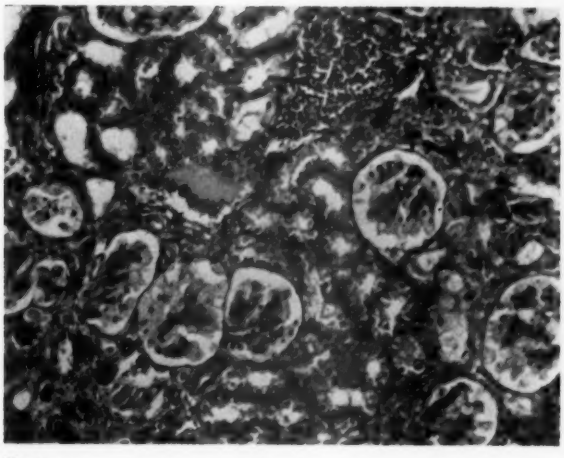


Fig. 9.—Kidney of rat with glomeruli composed of foam cells faintly bluish-stained (PVP).

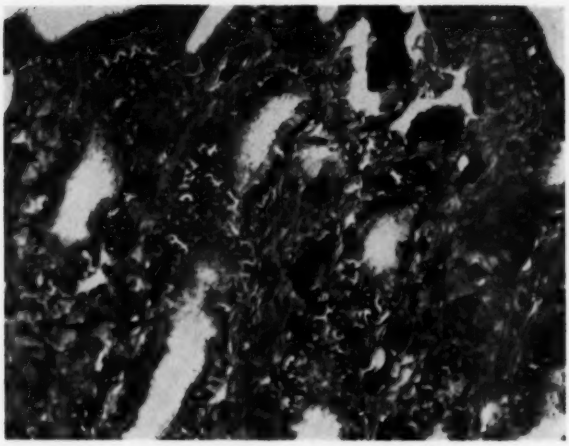


Fig. 10.—Endometrium of rat with interstitial foam cells and giant cells which are deeply blue-stained (PVP).

the spleen, where the pulp contained occasionally masses of huge multinucleated syncytia (Fig. 8), displaying a bluish-stained cytoplasm. "Lakes" of bluish matter apparently originating from a disintegration of reticulum cells were often observed in the bone marrow.

Blue-stained foam cells originating from capillary endothelium were noted in the renal glomeruli (Fig. 9). Bluish matter was contained also in tubular epithelial cells as well as tubular lumens. The tubules affected sometimes showed a hyperchromatic cell lining of syncytial character.

Massive deposits of PVP were often found in the endometrium, where they were located in clusters of foam cells as well as where they were infiltrating the intercellular spaces, thereby coming in close contact with the glandular epithelium (Fig. 10). Blue-stained histiocytes occurred frequently in the ovarian stroma.

It is evident from the evidence present that given adequate amounts of PVP this substance will be distributed diffusely in the body and will be retained in many organs and tissue not only in phagocytic cells but also in the parenchymal cells of several organs, such as the brain, liver, and kidney.

(*b*) Hyperplastic and Precancerous Reactions: Many of the organs and tissues in which PVP was stored displayed hyperplastic reactions which sometimes assumed

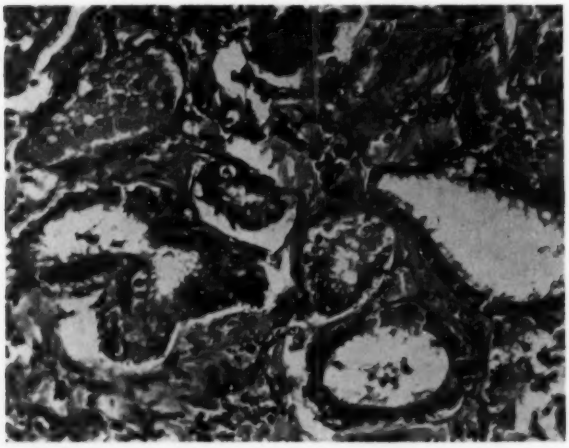


Fig. 11.—Alveolar adenomatosis of the lung in a rat (PVP intraperitoneally).

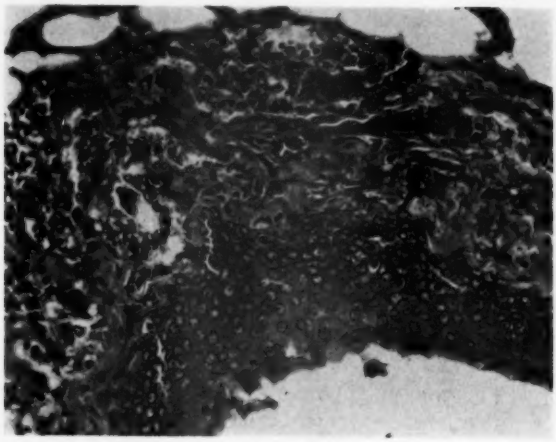


Fig. 12.—Bronchiolar cyst lined by a stratified squamous-cell epithelium with peribronchiolar adenomatosis in a rat (PVP intraperitoneally).

metaplastic as well as "preneoplastic" characteristics if standards of human cancerology are applied. The storage of PVP in ependymal, choroid, and meningeal cells was always accompanied by marked proliferations of the cells affected.

Multifocal adenomatosis of pulmonary alveoli, particularly in the peribronchial areas, was rather frequently encountered in rats (Fig. 11). In several rats these lesions were associated with the occurrence of multiple cysts lined by a stratified squamous epithelium and originating apparently from bronchial structures (Fig. 12). The pleura as well as the epicardium and endocardium of rats exhibited focal proliferations of closely packed hyperchromatic oval cells, which displayed a tendency to invade the underlying tissue and therefore became suspected of possessing potentially malignant properties or of representing actually early cancerous manifestations.

Similar diagnostic difficulties were met in assessing the potential and actual biologic character of the focal as well as diffuse proliferations of Kupffer cells in liver. These cells possessed sometimes a foamy cytoplasm, at other times they represented round or oval cells arranged often in ill-defined nodules or trabeculae (Figs. 13 and 14). Decisions concerning the benign or malignant nature of some of these reactions were by necessity sometimes of a rather arbitrary nature. The spleen and lymph

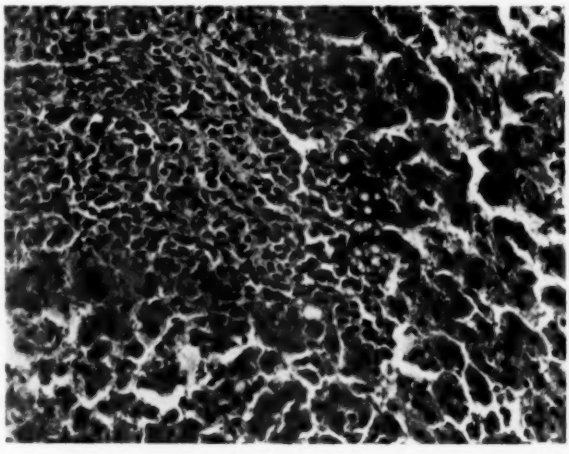


Fig. 13.—Focal Kupffer-cell proliferation in the liver of a rat (PVP subcutaneously).

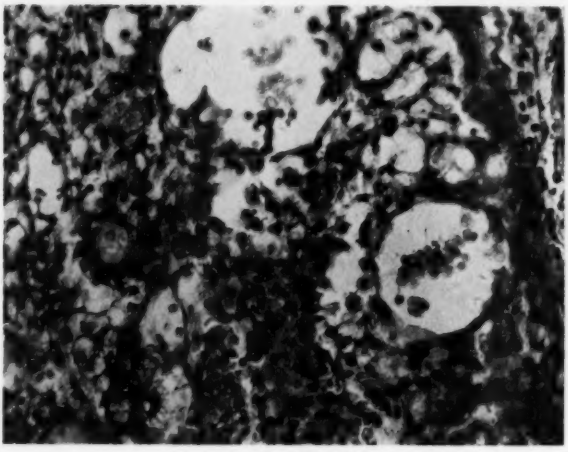


Fig. 14.—Focal Kupffer-cell proliferation of foam cell type in a rat (PVP).

nodes showed in some animals marked proliferations of reticulum cells and an occasional loss of follicular structure.

A marked glandular hyperplasia of the endometrial glands frequently assuming the character of a massive adenomatosis and associated with the formation of cysts, epithelial papillomatosis, and cellular atypia was noted in a considerable number of rats and rabbits. Such glandular proliferations were sometimes coexisting with focal or diffuse squamous-cell metaplasia of the endometrial epithelial lining as well as at times with areas of definite adenocarcinoma or squamous-cell carcinoma (Fig. 15). An unusual degree of cellularity of the endometrial stroma was also observed in some rats with PVP deposits.

Noteworthy in this connection is, moreover, the coexistence of large masses of blue-stained foam cells invading the adjacent muscular tissue located near an osteoid and possibly sarcomatous tumor involving the lower jaw in a rabbit (Fig. 16).

(*c*) Cancerous Reactions: The malignant nature of the lesions adjudged to be cancerous was attested not only by their histologic characteristics but also by their locally invasive growth, their diffuse involvement of the target organ, and often by the frequent presence of widespread metastases.

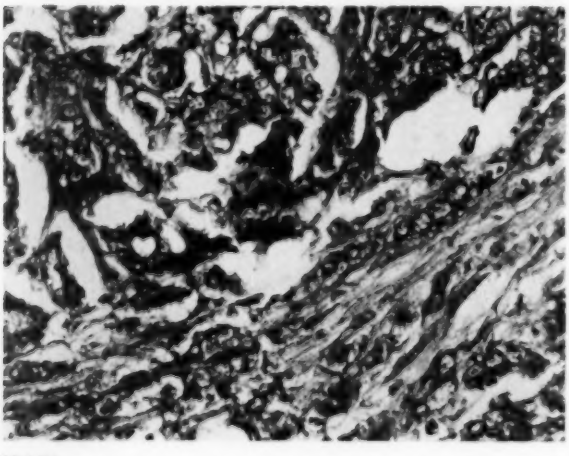


Fig. 15.—Adenocarcinoma of the uterus in a rat (PVP).

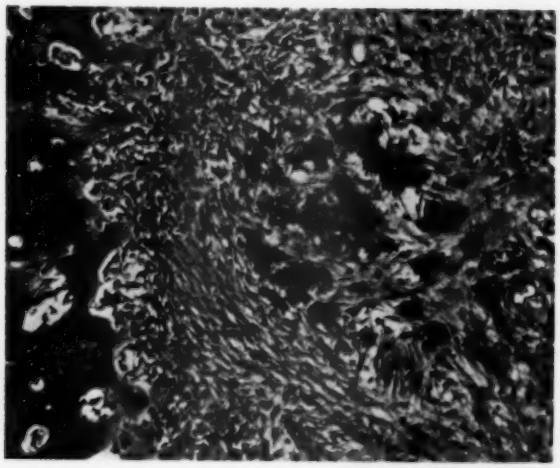


Fig. 16.—Sarcomatoid osteogenic tumor of the lower jaw with groups of phagocytic cells containing blue-stained material (PVP) (rabbit).

17. Endocardial mesothelioma in a rat (PVP).

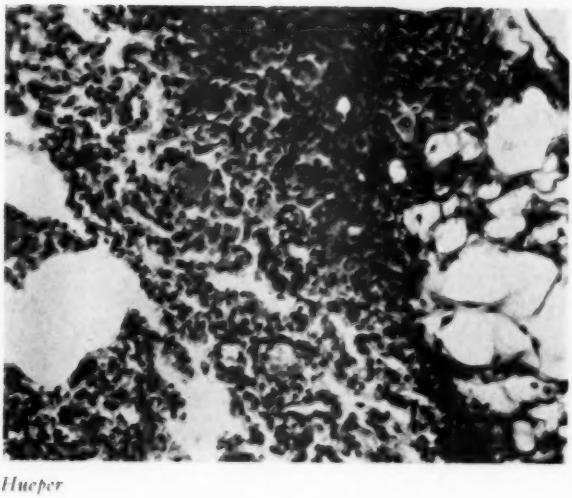


Fig. 18. — Marked mesothelial proliferation with foam cells of the pleura of probably sarcomatous nature in a rat (PVP).

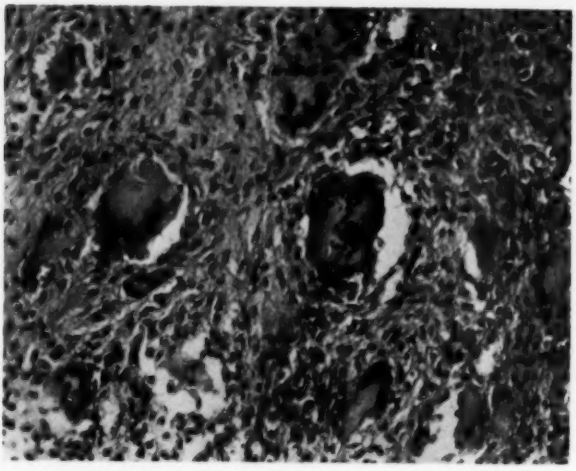


Fig. 19. — Kupffer-cell sarcoma with multinucleated giant cells of the liver in a rat (PVP).

The endothelio- or mesotheliosarcomatous neoplasms originating from the endocardial, epicardial, and pleural lining cells consisted of closely and irregularly packed oval cells invading the myocardium or lung, respectively. They extended along the pulmonary or cardiac vessels into the mediastinum and mediastinal lymph nodes and formed there large white medullary masses (Figs. 17 and 18).

The Kupffer-cell sarcomas displayed usually a similar cellular structure, being composed of strands or masses of oval-shaped cells or of irregularly round cells with a reticulum network. Huge multinucleated giant cells were present occasionally in these cell masses (Fig. 19). In addition to these sarcomatous hepatic lesions a few livers displayed changes of a leukemic character of monocytic or lymphocytic type.

Similar round cellular malignant neoplasms forming nodular masses in the peritoneal lymph nodes and invading the abdominal and thoracic organs apparently originating from the reticulum cells of lymph nodes and only occasionally from those of the spleen. The presence of giant cells in some of these tumors produced a resemblance with histologic changes found

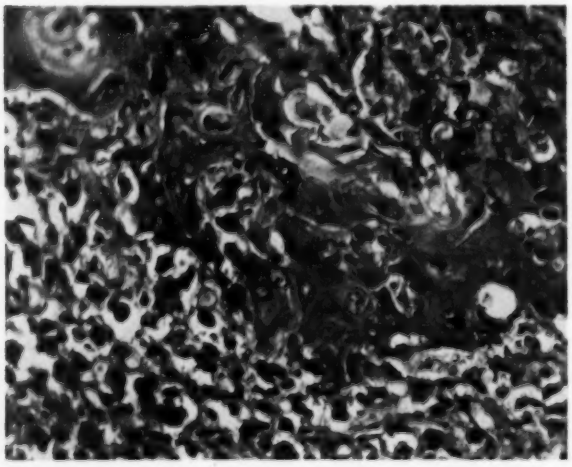


Fig. 20.—Squamous-cell carcinoma and round-cell sarcoma of the uterus of a rat (collision tumor) (PVP).

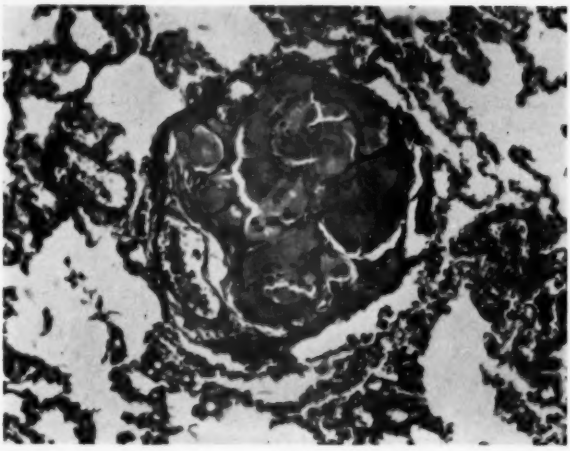


Fig. 21. — Intracapillary cluster of foreign-body giant cells in the lung of a rabbit (dextran).

in sarcomas derived from Hodgkin's disease in man.

The cancers affecting the uterus of some rats and of one rabbit were either adenocarcinomas or squamous-cell carcinomas or anaplastic carcinomas. Occasionally a mixture of adenocarcinoma and squamous-cell carcinoma or of adenocarcinoma and endometrial round-cell sarcoma was observed (Fig. 20).

Two rats developed squamous-cell carcinomas of the skin in the region of the subcutaneous implantation of PVP.

The histopathologic changes recorded for mice, rats, and rabbits which received PVP's by various parenteral routes demonstrate the topographical and in part also cellular identity of the localization of thesaurismotic, hyperplastic, and cancerous reactions. Statistical as well as histopathologic evidence thus support the view that at least some of the cancers observed in these animals are attributable to the PVP administered.

II. Polyvinyl Alcohol.—The storage phenomena observed were essentially identical in type and distribution with those seen in the PVP animals as well as with those previously reported for dogs and rabbits,[38,39] i. e., polyvinyl alcohol was present in vari-

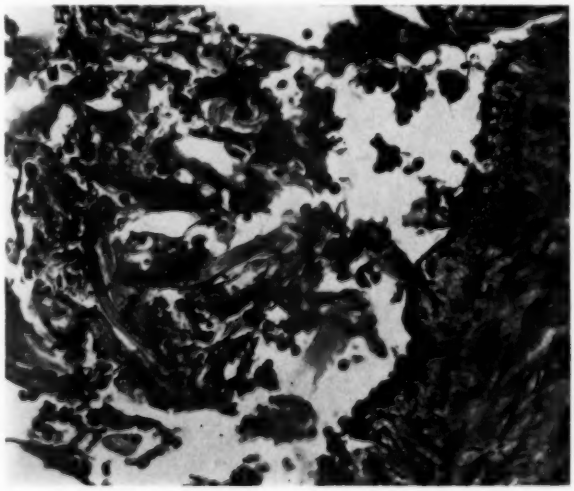


Fig. 22.—Foreign-body giant-cell granuloma obstructing the lumen of a large pulmonary artery in a rabbit (dextran).

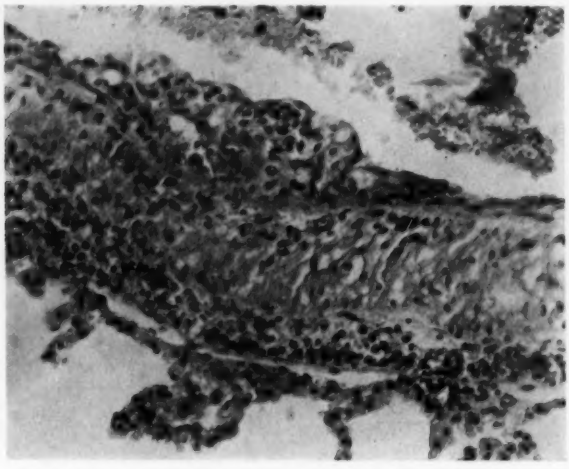


Fig. 23.—Intimal cushion of proliferated endothelial cells in a pulmonary artery of a rabbit (dextran).

ous cellular constituents, such as periportal foam-cell granulomas, isolated and swollen Kupffer cells, proliferated reticulum cells and giant cells of the spleen, and interstitial giant-cell granulomas of the lung. The cancerous reactions likewise resembled the corresponding ones observed among the PVP animals, i. e., Kupffer-cell sarcomas and mesotheliomas.

III. Dextran.—(*a*) Thesaurismotic Reactions: The lungs of rabbits revealed frequently intracapillary giant-cell granulomas, which were sometimes rather cellular and at other times mainly hyaline (Fig. 21). Similar but much larger formations occasionally blocked the lumens of large pulmonary arteries (Fig. 22). In the presence as well as in the absence of such foreign-body granulomas the endothelial lining of arteries exhibited sometimes focal increases of cells which at times produced small crescent-shaped, cellular plaques of the intima (Fig. 23). Apparently older lesions of this derivation appeared as fibrous intimal thickenings (Fig. 24).

Foam-cell accumulations in intrahepatic sinusoids were only occasionally observed in the livers of mice (Fig. 25). In the spleen of one rabbit hyaline masses surrounded atrophic lymph follicles. The same rabbit exhibited also an extensive hyalinosis of the renal glomeruli.

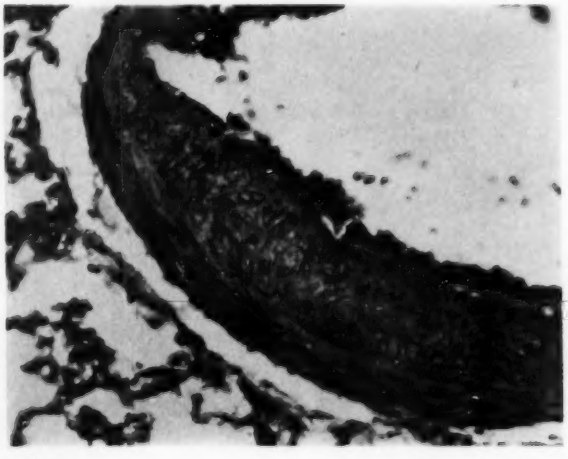


Fig. 24.—Fibrous intimal thickening in a large pulmonary artery of a rabbit (dextran).

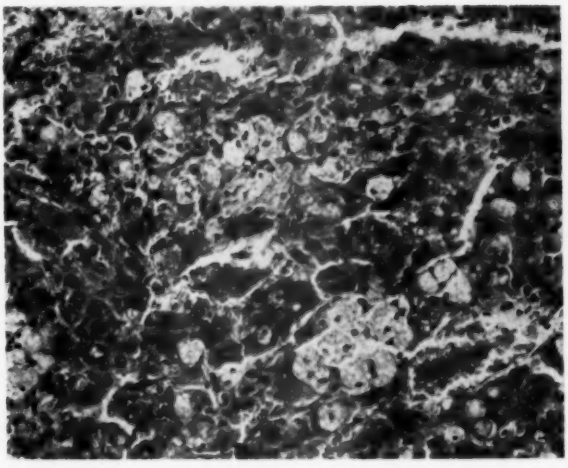


Fig. 25. — Proliferated Kupffer cells with swollen, foamy cytoplasm of a mouse (dextran).

The storage phenomena were in general distinctly less frequent and less marked in animals treated with dextran than in those given PVP's.

(*b*) Hyperplastic and Precancerous Reactions: The hyperplastic and precancerous reactions were correspondingly also less marked and less frequent in dextran animals than in PVP animals. The spleens of some mice and of one rabbit revealed marked mononuclear or reticulum-cell proliferations obliterating the lymph follicular structure of this organ. Two mice showed, moreover, pulmonary adenomas, one of which projected above the pleural surface as a polypous formation (Fig. 26). The lung of one rabbit exhibited a large area of alveolar adenomatosis.

(*c*) Cancerous Reactions: The cancerous reactions in dextran animals resembled in histologic type and in topographical distribution those seen in PVP animals. The various types of cancers found in dextran-treated animals were endothelioma of the endocardium (Fig. 27), myeloid leukemia, reticulum-cell sarcoma of the liver or of the lymph nodes, and carcinoma or carcinosarcoma of the uterus. It is noteworthy that

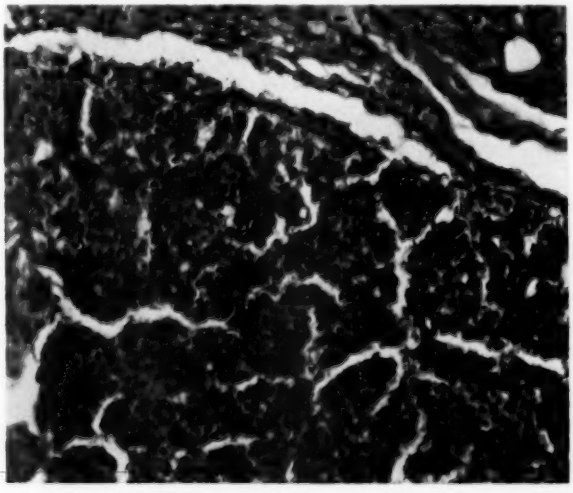


Fig. 26. — Polypous adenocarcinoma of the pleura of a mouse (dextran).

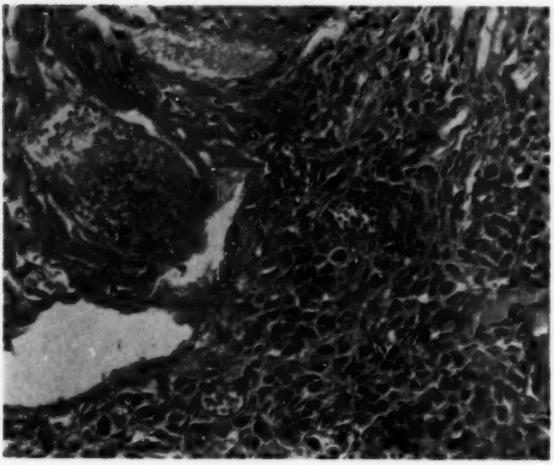
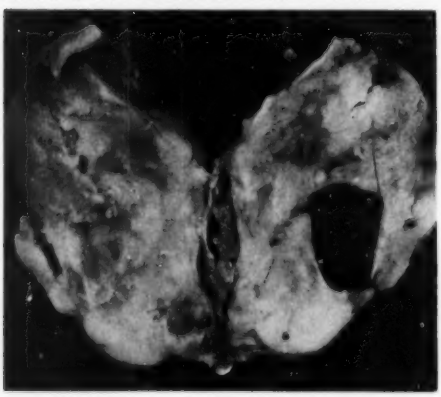


Fig. 27. — Pericardial endothelioma invading the myocardium of a rat (dextran).

this identity in histogenesis and histological structure of the cancers seen in dextran and PVP animals is shared only in part with those seen in normal control animals, which, while carrying some of the cancers recorded in the experimental animals, show them at a considerably lower incidence rate and lack also the various and frequent developmental and "precancerous" stages seen in the test animals.

IV. Silastic Rubber.—This observation applies also to the cancers found in the internal organs of rats which received implants of silastic rubber or gum into the subcutaneous tissue or into the abdominal cavity. These tumors occurred in these experimental animals at a normal frequency rate. The spindle-cell sarcomas which developed around the subcutaneously implanted cubes of silastic rubber, on the other hand, represent specific reaction products to the processed silicon polymer (Fig. 28). The histologic examination of the capsules surrounding these implants in the subcutaneous tissue and in the abdominal cavity revealed that they consisted originally of oligocellular hyaline membranes which were thicker for implants in the subcutaneous tissue than for those in the abdominal cavity. In some capsules multicentric small cellular foci were found beneath the inner lining which were not in direct contact with the implant. It may be assumed that the subsequent sarcoma formation started from these foci.

Fig. 28.—Fibrosarcoma surrounding a cube of Silastic in the subcutaneous tissue of a rat.



It is remarkable that none of the capsules formed around the abdominal implants developed sarcomas and that such an event was observed in only one of two series of 30 rats each which received implants of the silicon latex. While tumors were not formed around the implants of powdered silica placed in the subcutaneous tissue and into the peritoneal cavity, the relatively large number of cancers of the internal organs observed in rats of these series creates the suspicion that the two phenomena may not entirely be unrelated.

Mention may finally be made of the fact, which is apparent from the descriptions of the histological changes observed in the different experiments, that storage phenomena and hyperplastic reactions were present in animals with and without neoplastic reactions. In these respects animals with tumors did not differ as a group from animals without tumors.

Comment

The evidence presented indicates that under the experimental conditions observed some, but not all, PVPs; some, but not all dextrans; a polyvinyl alcohol, and Silastic are carcinogenic to mice, rats, and perhaps also to rabbits, when they are introduced parenterally. The existence of fundamental differences in carcinogenic properties between polymers of the same type, such as PVPs, was suggested by the divergent results reported previously, since Hueper and Nelson and Lusky obtained sarcomas with PVP introduced into rats, while Hecht failed to do so.

The carcinogenic effect of the water soluble polymers involves several organs and tissues. It becomes manifest mainly in organs in which these macromolecular substances are retained and stored over prolonged periods and where they tend to elicit reactive cellular proliferations which seem to supply the preparatory state for the subsequent development of cancers. In contrast, the carcinogenic effect of water insoluble polymers whenever it is present is limited to the site of implantation, i. e., mainly the subcutaneous connective tissue.

Differences in carcinogenic potency between polymers of the same type and of different types seem to be reflected in variations in the length of the minimal latent period. The minimal preparatory period of cancers for PVPs 1, 2, 3, and 4 was six to eight months. Since about 25% of all cancers among the rats of this experiment occurred during the 7th to 12th months of the exposure time, these particular PVPs exhibited a considerable degree of carcinogenic potency as judged by the length of the minimal latent period. Subsequently studied carcinogenic PVPs, dextrans, polyvinyl alcohol, and silicon rubber, on the other hand, elicited cancers not until the elapse of at least one year following the start of the experiment. A complete lack of carcinogenic response during the first 12 to 15 months of observation has been noted so far for the different polyethylene and polyurethane preparations parenterally introduced by various routes and in different physical forms.

The route of administration which apparently influences the time of onset and the degree of development of fibrous encapsulation of the implanted materials and its relative concentration in the tissues seems to exert a determining influence upon the degree of the carcinogenic effect of water-insoluble polymers, since sarcomas developed at the site of encapsulation of water insoluble polymers much more often in the subcutaneous tissue than in the peritoneal cavity. There was, on the other hand, no consistent difference in the carcinogenic response in internal organs for the various routes of introduction of water-soluble polymers.

When the degrees of carcinogenic response obtained with water-soluble polymers of different molecular size and shape were compared, again no distinct pattern of behavior was discernible. This lack of distinction extended also to reactions elicited by products of commercial grade and those of medicinal grade. The results of experiments with PVP 6 and PVP 7 which were administered in one set in single doses and in a second set in repeated doses suggested, however, that repeated exposures intensified the carcinogenic effect and thus brought to light weak carcinogenic properties which were not apparent for PVP 7 after the introduction of a single dose.

These observations are of fundamental importance because they indicate that the differences in carcinogenic behavior of different polymers of the same general type

are apparently related to much more complex differences than those represented by mere variations in average molecular weight or the ratio of branched to linear member groups in a polymer. The marked carcinogenic effect of the Silastic molecule possessing cross linkages of peroxide nature in comparison to the at best weakly carcinogenic one of the linear latex molecule provides perhaps a clue to a future study of this intricate problem. It is noteworthy in this connection that Mark,[40] working with polyvinyl macromolecules, recently pointed out that certain polymers can have different properties if they have been prepared under different conditions, not because of head-to-tail, head-to-head isomerism and not because they represent different degrees of branching, but simply because the substituents are differently arranged in space as one progresses along the chain and affect the configuration of the chain as a whole. It is, therefore, possible that the industrial use of different catalysts which determine the character of the end-groups and of different production methods in the preparation of polyvinylpyrrolidones may account for the differences observed in carcinogenic potency of PVPs of different manufacture (PVP 2 and PVP 7 of German manufacture; PVP 1 and PVP 6 of American origin).

Support of this concept of a chemospecific basis for the carcinogenic action of polymers and macromolecular condensates is provided by the recent observation of Richmond,[41] showing that an iron-dextran complex when parenterally introduced into rats was carcinogenic, while the dextran alone was not. A similar connotation may be attached to the finding that the carcinogenic polyamide "Perlon" (e-amino-caprolactam), when used as a filling of human pleural cavities, undergoes gradual disintegration of its fibrils and that in fact the fibrils varied structurally when viewed under the microscope before they were implanted. This observation indicates that at least some polymers (Mondur TD-80) do not seem to be stable in a biologic medium; nor are they chemically uniform. These facts may account for the variability of the anatomic reactions to various Perlon products, since some elicit a marked foreign-body reaction and fibroblastic proliferation, while others seem to be almost inert in this respect (Wolter).

The action of a chemical mechanism in the production of anatomic reactive lesions to polymers is, moreover, suggested by the development of granulomatous reactions in the lungs of man following the inhalation of PVP-containing hair lacquers (Bergmann, Flance, and Blumenthal) and of similar pulmonary changes in rats after the intratracheal injection of dust of Perlon and Nylon (polyhexamethylene adipamid [Massmann and Pilgrim]). The pulmonary reactions in rats were progressive and later on complicated by the development of extensive adenomatous hyperplasias originating from bronchial epithelium. Although these adenomatous responses were considered by the investigators as unrelated to the inhalation of the polyamides and although cancerous pulmonary reactions were not observed, these findings nevertheless show that some finely dispersed and powdered polymers exert distinct biologic effects in the organs of contact which must be ascribed to chemical interactions and not to the action of mere surface forces. There is no sound reason for assuming that the marked fibrosing and scar tissue development stimulating action of polyethylene films utilized in human surgical practice has any other than a chemical basis (Yeager and Cowley). The serious toxic reactions, such as alopecia, thrombocytopenia, diarrhea, and anorexia, elicited by dextran sulfate in man, are doubtlessly responses to chemical phenomena (Tudhope et al.). In fact, Tudhope compares these effects of dextran sulfate with those produced by whole-body irradiation and by exposure to mechlorethamine hydrochloride (nitrogen mustard), colchicine, and other antimitotic substances and thereby refers to chemicals and physical agents which often have ambivalent qualities, i. e., they are also carcinogenic under proper conditions.

There can be no doubt that some water-soluble as well as water-insoluble polymers are capable of producing in the organism of man and experimental animals toxic, proliferative, and even carcinogenic reactions, which in their general behavior and type cannot be distinguished from those elicited by other chemicals. The fact that evidently only some but not all polymers of the same general type seem to be carcinogenic has its well known analogue in the observation that only some and not all derivatives of certain polycyclic aromatic hydrocarbons or of aromatic amines or of azo compounds are carcinogenic and that often some specific and often apparently minor aspect of their chemical composition as well as molecular configuration are the determining factors in this respect.

Apart from a direct chemical interaction of these polymers, particularly the water-soluble variety, with cellular constituents, there exist two additional mechanisms through which these metabolically rather inert macromolecules may act upon cells. Their intracellular and extracellular storage in excessive amounts doubtlessly interferes in the long run with the normal vital cellular activities by hindering intracellular and extracellular reactions and fluid and chemical exchange processes, thereby causing cellular degeneration and death. These, in turn, may be followed by reactive regenerative cell proliferation. Such possible effects may involve the blood-brain barrier, owing to the accumulation of PVP in the choroid plexus, meninges, and adjacent cerebral tissue, and thereby impair cerebral function. They may affect the nutrition of the arterial walls as well as the liver and kidney function. Storage of PVP in the spleen, resulting in secondary reticulum-cell proliferation, may exert an influence upon erythrocytic destruction rate and thereby account for the delayed appearance of anemia often developing as a manifestation of hypersplenism in excessively stored animals (macromolecular hematic syndrome).

Since PVP has a definite affinity to form complexes with proteins, dyes, various drugs, and other chemicals, i. e., it exerts an "embathic" effect, its marked accumulation in the endometrium raises the question whether it has also an affinity to sex hormones and may elicit through the mechanism of local hyperestrogenism, endometrial hyperplasia, and carcinoma.

Concerning the merits and the validity of the theory on carcinogenesis by surface action and capsule formation of polymer films, which is also applied to the carcinogenic action of metal foils, it may be pointed out that cancers can readily be produced in rats by the implantation of certain powdered metals, such as uranium and nickel, and have been produced also in rabbits with chromium powder (Schinz). Since similar cancerous responses can also be elicited by the subcutaneous implantation of certain metal compounds, such as calcium chromate, in rats or by the intravenous injection of beryllium compounds into rabbits, it is very certain that the process of metal carcinogenesis does not depend on any nonspecific physical phenomenon related to surface forces but is attributable to specific chemical effects of these metallic elements and their proper biologic availability to the tissues.

It is, moreover, obvious that the carcinogenic effect of water-soluble polymers is not dependent upon the presence of a certain number of square millimeters of unbroken surface of polymer film.

The mere formation of a capsule around implanted polymer films also cannot be considered as the specific prerequisite for a subsequent cancerous development, since the balls of silicon latex subcutaneously implanted were as effectively encapsulated as the cubes of silicon rubber. Nevertheless, sarcomas mainly developed around the rubber cubes and not around the latex balls, indicating that cancerous responses in the capsule tissue depend on an additional and specific carcinogenic factor associated with the polymer. If indeed the formation of cellular scar tissue in rats would represent a fertile basis of the production of cancers, it is surprising that the chronic organizing

pneumonias not infrequently seen in rats do not seem to give rise to carcinomas or sarcomas of the lung, unless a specific carcinogenic agent is active. While at the present time a satisfactory explanation for the differences in cancerous responses to various polymers when implanted as intact film, perforated film, fabric, thread, or powder cannot be given, it is most unlikely that for the reasons given the hypothesis of Nothdurft and of Oppenheimer on polymer carcinogenesis is the correct one.

Finally, comments are in order concerning the applicability of the experimental observations to man, i. e., whether or not health and cancer hazards may result from any exposure to the growing number of synthetic and semisynthetic macromolecular substances for occupational or medicinal reasons or as the result of handling, using, or ingesting the large number of consumer goods either containing these chemicals or having been in contact with them.

The various storage phenomena produced by water-soluble polymers which have been observed not only in experimental animals but for the plasma extenders, especially PVP, also in man (Busch, Traenckner,[29] Gropper et al., Hartmann and Behrmann,[28] Wrage, Upham et al., Ravin et al.) in general have been considered as of little consequence as to any acute or chronic effects upon the function of the organs affected. This conclusion is probably correct unless highly excessive amounts of these agents, particularly PVP, are administered repeatedly over prolonged periods of time. The observations of the present experiments reported add to the list of already known thesaurismotic reactions from which possible delayed health hazards may result under exceptional conditions. These are the storage phenomena in the choroid plexus, the glia and ganglion cells of the brain, and the atheromatous and atherosclerotic arterial reactions seen in rabbits given injections of PVP and dextrans. If these observations indicate that the medicinal and cosmetic use of water-soluble polymers should not be practiced without caution and discrimination, this warning receives increased emphasis by the demonstration of carcinogenic effects elicited by both water-soluble and insoluble polymers. While there exist without any doubt justifiable and legitimate medical and surgical indications for the use of such macromolecular agents, the present uncertainty of their potential carcinogenic action in man upon parenteral or respiratory introduction demands that the calculated risk related to the disease to be treated outweighs definitely any possible delayed risk to health which may be created by a polymer administered.

Although at present no valid evidence exists that the use of such polymers in certain protheses, such as dentures and contact lenses, which have prolonged and intimate contact with mucous membranes, is actually related to an increased occurrence of cancer in the exposed tissues, it seems to be wise to conduct during the coming years carefully controlled surveys on the cancer incidence among persons thus exposed for determining the actual innocuousness of these plastic devices.

Similar considerations may well be extended to the medicinal use of ion-exchange resins (polyamine-methylene resin, carbacrylamine resin) administered by mouth for increasing the fecal excretion of sodium in cardiac edema or as an antacid in the treatment of gastric hyperacidity. Definite attention should be given in this respect also to the incorporation of macromolecular chemicals into other medicinal and cosmetic products, such as ointments, creams, and laxatives. Although the scientific character of the experimental evidence of the carcinogenesis of polymers does not justify any undue alarm concerning the use of such materials in food products, for the impregnation or coating of food containers, or for the coating or wrapping of foodstuffs, it appears to be a sound precautionary measure to keep all workers employed in the production, processing, and handling of polymers or plastics under proper medical supervision for insuring an early discovery

of any cancers possibly attributable to contact with these substances.

Conclusions

1. Certain water-soluble polymers, such as polyvinylpyrrolidone, polyvinyl alcohol, and dextran elicit upon parenteral introduction into rats and mice, and perhaps also in rabbits, sarcomas originating from organs and tissues in which these macromolecular substances are retained and stored, i. e., the reticuloendothelial tissues. Cancers from other tissues, such as uterus and skin, observed in rats may be directly or indirectly caused by these substances.

2. Polymers of the same general type but produced by different procedures and varying among each other in molecular weight and configuration differ greatly in their carcinogenic potency, i. e., some are noncarcinogenic, others weakly carcinogenic, and again others definitely carcinogenic.

3. The experiments conducted have failed so far to provide any information on the physical or chemical molecular factors which may account for these differences.

4. Experiments with silicon rubber and silicon latex showed that the rubber having a net molecule with many cross linkages possesses marked carcinogenic properties when subcutaneously implanted into rats, while the latex having a linear molecule is at best weakly carcinogenic.

5. The total evidence on hand concerning the biologic action of synthetic and semisynthetic polymers parenterally introduced into man and animals favors the view that their toxic, pharmacologic, and carcinogenic effects are mainly related to chemical properties and reactions rather than to their physical characteristics.

6. The existence of actual cancer hazards to man from water-soluble and insoluble polymers possibly associated not only with their parenteral introduction but also with their administration by the cutaneous, respiratory, and oral routes is at present problematical. The experimental evidence on hand, however, is sufficiently definite and serious for requiring distinct caution in the medicinal and cosmetic use of these products.

The various dextrans were supplied by Mr. H. S. Paine, The Dextran Corporation; Dr. H. E. Stavely, Commercial Solvent Corporation, and Dr. C. R. Rist, Starch and Dextrose Division, Bureau of Agricultural and Industrial Chemistry, Northern Regional Research Laboratory. The polyvinylpyrrolidones employed were furnished by Dr. B. M. Lanman, Schenley Laboratories, Inc.; Dr. J. Werner, General Aniline and Film Comporation; Prof. Dr. H. Oettel, Badische Anilin and Soda Fabrik; Dr. Beuchelt, Farbenfabrik Bayer, A. G., and Dr. H. Campbell, May and Baker, Ltd. Mondur TD-80 was supplied by Mr. J. H. Saunders, Mobay Chemical Company; polyethylene, by Dr. Th. Nale, Carbide and Carbon Corporation; polyvinyl alcohol, by Mr. R. H. Beeman, E. I. DuPont de Nemours and Company, and Silastic and silicone gum, by the Dow Corning Corporation.

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The Early Lesions of Aortic Atherosclerosis in Cali, Colombia

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The study to be reported here represents one part of a continuing cooperative project between several pathology laboratories, which has as its purpose to write the natural history of atherosclerotic arterial lesions for different population groups in different geographic regions. Data of this same type derived from necropsy populations of New Orleans, Guatemala, and Costa Rica have been reported previously,[1,2] and these figures have been used as a basis for comparison of the material collected in Colombia with that from New Orleans and Central America. The extension of this study to Colombia has been of particular interest in view of the fact that Colombia, according to vital statistics, has one of the lowest death rates from arteriosclerotic heart disease in all of the world.[3] This rate, even when adjusted for age distribution, amounts to approximately 0.1 of the rate for the United States and 0.5 of the rate for Guatemala.

This report is concerned only with aortic atherosclerosis in persons from 1 to 40 years of age. Similar data on coronary atherosclerosis, obviously more directly related to coronary heart disease, are being accumulated, but at a much slower rate.

Materials and Methods

Aortas were collected from consecutive necropsies in the Hospital Departmental Universitario of Cali and from the Oficina Medico-Legal of Cali. These sources are as close counterparts of the sources of the material in New Orleans and Guatemala as can be found. The Hospital Departmental Universitario is a teaching hospital, admitting patients mostly from the lower income groups. It does have private rooms which serve the upper income groups, but deaths from this group rarely come to necropsy. For example, in the calendar year 1957, only 7 out of the total of 304 autopsies came from the private rooms.

The aortas were opened longitudinally, formalin-fixed in the flattened state, stained grossly with Sudan IV, and packaged individually in transparent plastic bags.[4] The atherosclerotic lesions were evaluated by estimating the percentage of intimal surface involved by each of three different types of lesions: (1) fatty streaks, slightly elevated sudanophilic intimal plaques; (2) fibrous plaques, translucent gray elevated plaques not stained with sudan grossly but containing lipid beneath a layer of fibrous tissue, and (3) complicated plaques, those showing hemorrhage, ulceration, thrombosis, or calcification. The definitions of lesions, the criteria for evaluating them, and the methods of recording and analyzing data described in the report on the New Orleans material were closely followed. The present estimations of percentage of intimal surface involved by the lesions were made by averaging our individual estimates.

The Table shows the distribution by age and sex of the 289 cases for which essentially complete aortic specimens were available.

Early Lesions of Aortic Atherosclerosis, Distribution by Age and Sex

Age Group, Yr.	Males	Females	Total
1–5	14	21	35
6–10	7	9	16
11–15	9	4	13
16–20	24	21	45
21–25	24	19	43
26–30	25	17	42
31–35	29	22	51
36–40	22	22	44
Total	154	135	289

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From the Departments of Pathology of the Facultad de Medicina, Universidad del Valle, Cali, Colombia, and Louisiana State University School of Medicine, New Orleans.

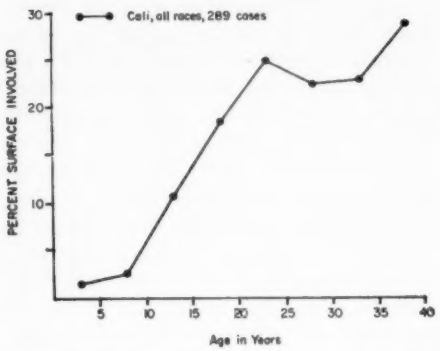

Fig. 1.—Aortic atherosclerosis in Cali; fatty streaks.

Results

Fatty Streaks.—Figure 1 shows the variation with age of simple fatty streaks in this group. Practically all of the specimens had minimal lesions by the age of 3 years, but they covered only 1.6% of the total intimal surface. By 8 years of age every aorta without exception was involved, but the extent of fatty streaks has risen to only 2.6%. After 8 years of age the surface involved with fatty streaks began a rise, which continued steadily until age 23, reaching a peak of 25%. Thereafter the growth of fatty streaks levels off and actually drops slightly. By 40 years the surface involvement with fatty streaks has risen again to 30%.

Figure 2 shows this same curve for Cali, superimposed on similar lines drawn for New Orleans Negroes, New Orleans whites, and Guatemalans, the figures for which were taken directly from previously published data.[1,2] The striking feature of this combined graph is that the progression of fatty streaks with age in the young Colombians follows a pattern very similar to that of the other groups. During the second decade it occupies a position intermediate between the New Orleans Negroes on the one hand and the New Orleans whites and Guatemalans on the other. The peak of severity, the subsequent drop, and the later rise in the fourth decade occur several years later but parallel the course of the fatty streaks in the New Orleans Negroes.

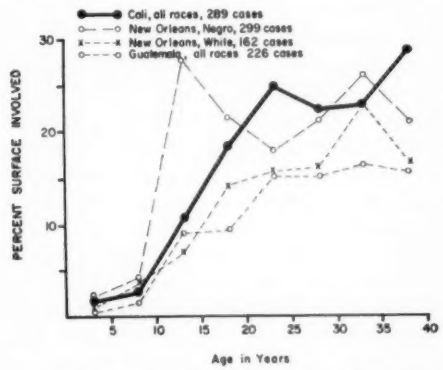

Fig. 2.—Aortic atherosclerosis in Cali, New Orleans, and Guatemala; fatty streaks.

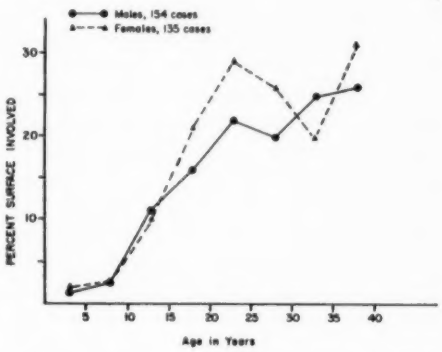

Fig. 3.—Aortic atherosclerosis in Cali; fatty streaks.

Some of the apparent differences between the four groups at certain ages were tested for statistical significance. In the 11- to 15-year-old group the difference between New Orleans Negroes and the Colombians was highly significant (P, less than 0.01). In the 21- to 25-year-old group the Colombians have significantly more fatty streaks than the New Orleans whites or Guatemalans (P, less than 0.01). In the 36- to 40-year-old group the Colombians again have more fatty streaks than both races from New Orleans and the Guatemalans.

Division of the Colombians by sex with respect to the development of fatty streaks gives the relationship shown in Figure 3. Males and females are involved to almost exactly the same degree at most ages, except

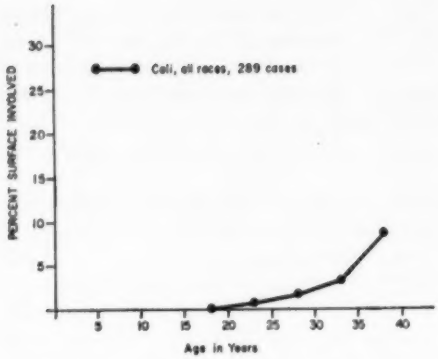

Fig. 4.—Aortic atherosclerosis in Cali; fibrous plaques.

that females have somewhat more extensive fatty streaks between 15 and 30 years. This increased involvement with fatty streaks in females was not statistically significant in any one age group, but in the three age groups together it was significant at the 5% level. Reference to the data from the other three population groups will show that in this respect the Colombians are very similar to the New Orleans Negroes. It was suspected that perhaps the high maternal mortality in this age group in Colombia might have been responsible for this increased extent of fatty streaks, but reexamination of the individual cases yielded no correlation between pregnancy or the postpartal state and the severity of fatty streaks.

Fibrous Plaques.—Figure 4 shows the average degree of percentage surface involved with fibrous plaques in the Colombians, and Figure 5, the comparison of the Colombians with the other groups. The fibrous plaques in the Colombians follow a pattern very similar to that seen in the New Orleans population but lower than the whites and about equal to the Negroes. On the other hand, the involvement is definitely higher than in the Guatemalans. The variation in fibrous plaques is so great that none of the differences between the Colombians and the others are significant at the 1% level, but the trend of the Colombians appears to be consistent.

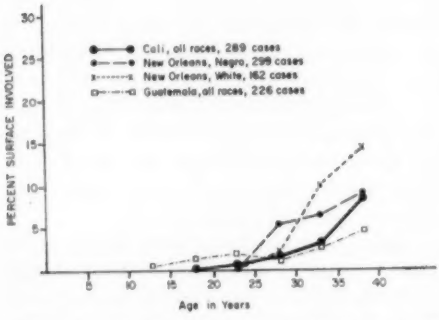
Cali, all races, 289 cases
New Orleans, Negro, 299 cases
New Orleans, White, 162 cases
Guatemala, all races, 226 cases
PERCENT SURFACE INVOLVED
Age in Years

Fig. 5.—Aortic atherosclerosis in Cali, New Orleans, and Guatemala; fibrous plaques.

Complicated Lesions.—As with the other groups, in the age period under 41 the incidence of aortic lesions with hemorrhage, ulceration, thrombosis, or calcification was so infrequent that these were quantitatively insignificant in terms of percentage of surface involved.

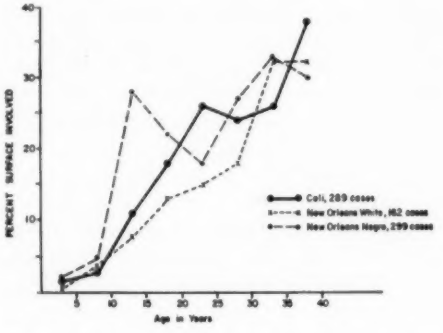

Fig. 6.—Aortic atherosclerosis in Cali vs. New Orleans; fatty streaks and fibrous plaques.

Total Percentage of Surface Involved.—Figure 6 shows the comparison of the total percentage of aortic intima involved with lesions (fatty streaks plus fibrous plaques) with the other population groups. Although in the 36- to 40-year age group the Colombians are slightly more involved, recall that fibrous plaques make up a much higher proportion of the lesions in New Orleans whites than in the Colombians or New Orleans Negroes. Consequently this figure of "total percentage of surface involved" does not reflect accurately the actual severity of the atherosclerotic process. However, in

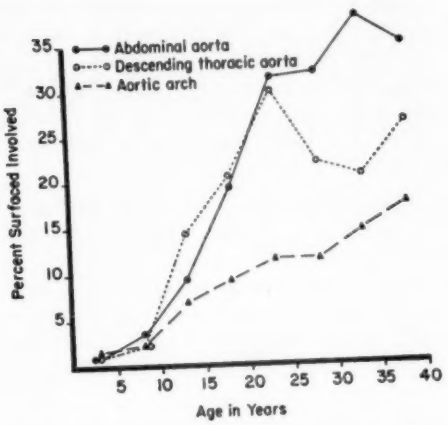

Fig. 7.—Aortic atherosclerosis in Cali; fatty streaks, by anatomic region.

view of our ignorance of the exact relationship between fatty streaks and fibrous plaques, we believe that it is premature to assign an arbitrary value to weigh each type of lesion.

Comparison of Traumatic Versus Natural Deaths.—There was a sufficient number of cases of sudden or rapid death due to trauma to make such comparisons only in males in the age groups from 16 to 40 years of age. In these groups there was no significant difference between the average extent of fatty streaks in persons dying of various acute and chronic diseases in the hospital and those dying suddenly of accidents, wounds, or poisoning, just as was found in the study from New Orleans and Guatemala.

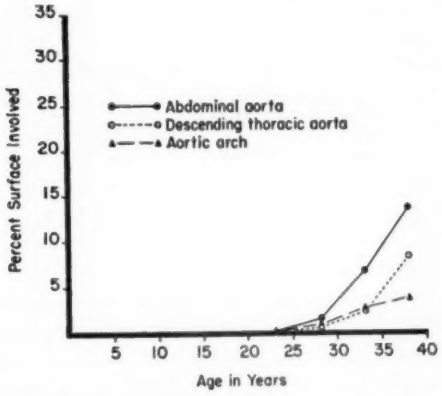

Fig. 8.—Aortic atherosclerosis in Cali; fibrous plaques, by anatomic region.

Extent of Lesions by Anatomic Region.—Figure 7 shows the percentage of surface involvement of each of three anatomic regions of the aorta with fatty streaks, and Figure 8, the involvement with fibrous plaques. There was not a sufficient number of specimens with the aortic ring included to draw a similar line for this portion, but, just as in the other populations, the ring was often the only portion with lesions in young children. The relative involvement of the various parts with both fatty streaks and fibrous plaques is practically identical to that encountered in the other three groups.

Comment

Before discussing the significance of these observations it would be of interest to describe the population from which the Colombian specimens were drawn. The lower income group, which practically all these cases represent, cannot be classified accurately as to race. The majority of them are referred to as mestizo or mulatto, representing a mixture between the Spanish immigrants, African Negroes, and indigenous Indians. In this part of Colombia there are practically no pure Indians, but there do remain some relatively pure Negroes. The cases in this study included only five patients recognized as pure or nearly pure Negroes, too few to analyze separately. The population of Cali is not representative of Colombia as a whole, for in other areas the population is predominantly pure white; in others, predominantly Indian, and in still others, predominantly Negro.

The climate of this area is subtropical, with a mean temperature the year around of 75 F and an altitude of 3,000 ft.

No accurate figures are available for the dietary consumption of the lower income groups in this population, but it is the consensus that their diet is low in protein and fat, high in carbohydrate, and on the bor-

derline in calories. Infant malnutrition of the kwashiorkor type is common. Nodular goiter is very common, and some anatomic abnormality of the thyroid gland is practically universal.

These characteristics illustrate just a few of the factors which must be taken into account in making such geographic comparisons. For example, between the three geographic regions (New Orleans, Guatemala, and Cali) we have not just three different environments with differing diets, but we also have at least four different racial groups, one of which is greatly hybridized. Many factors other than diet may be responsible for any differences which may exist in the degree of aortic atherosclerosis.

A hazard involved in the type of sample which is available for such a study is the selection of cases first by hospitalization, then by death, and finally by necropsy. Policies with respect to admissions and necropsy permissions may vary, and the causes of death may vary. For example, the cases from Cali include among the females from 16 to 30 years of age a much larger proportion of maternal deaths than those from New Orleans. However, the similarity in the extent of lesions in cases of sudden death due to trauma to that in persons dying of so-called natural causes leads us to believe that (at least in persons under 40 years of age) this hazard is not as serious as would be expected.

One of the most striking features of these data is the similarity of the pattern of development of the fatty streaks in the Colombians to that in the other groups, particularly of the New Orleans Negroes. The Colombians show the same rapid rise in the second decade, reaching a peak with subsequent regression about 10 years later than the New Orleans Negroes. Other characteristics in which the Colombians resemble the New Orleans Negroes are the greater involvement with fatty streaks of females than males in the 15- to 30-year age groups and the similarity of the average extent of fibrous plaques attained at age 40. It is an attractive hypothesis to suppose that this behavior of fatty streaks in Cali is due to the mixture of Negro blood with the white and Indian blood, but further evidence to substantiate such an explanation is lacking at the present time.

The rapid rise in fatty streaks during the second decade of life appears to be well established for the Colombians as well as for the other groups and provides additional data consistent with the hypothesis that the development of these aortic fatty streaks is a nearly universal phenomenon in the human species and possibly is related to the changing patterns of hormonal activity taking place during puberty. Furthermore, we have encountered in this population still another discrepancy between the degree of fatty streaks developing early in life and the degree of fibrous plaques developing subsequently. This finding again supports the hypothesis that the factors responsible for the fibrous plaques may be and probably are different from the factors responsible for the fatty streaks.

Data are not yet available on a sufficient number of coronary arteries from any of the population groups discussed here to make comparisons on the basis of coronary atherosclerosis proper. However, if there is a positive correlation between aortic lesions and coronary lesions and if it is true that advanced atherosclerotic lesions predispose to clinical atherosclerotic heart disease, then these data are hardly compatible with such a low rate of "arteriosclerotic heart disease" as is indicated for Colombia by vital statistics. Therefore, we will predict that the incidence of atheriosclerotic heart disease in Colombia as shown by vital statistics will ultimately be found to be in error, at least for the region around Cali from which this sample was drawn. More specifically, this rate should approximate that of the Negro population of New Orleans and be intermediate between that of the United States white population and that of Guatemala.

Summary

The aortas of 289 persons between 1 and 40 years of age from a large charity hospital and a medicolegal service in Cali, Colombia, were quantitatively evaluated for the extent of the various types of atherosclerotic lesions. These figures were then compared with similar data for New Orleans Negroes, New Orleans whites, and Guatemalans.

Fatty streaks increase rapidly in extent during the second decade in the Colombians, just as in the other groups, and the hypothesis that this development of fatty streaks is related to the hormonal changes of puberty is supported. Despite having more fatty streaks in the third decade, the Colombians develop fewer fibrous plaques than the New Orleans whites, suggesting again that different factors control the progression of the different types of lesion.

In view of the presumed relationship between lesions and clinical disease, these data on aortic lesions are not consistent with such a low rate of arteriosclerotic heart disease as is indicated by vital statistics for Colombia.

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Reduplication, Remodeling, Regeneration, Repair, and Degeneration of Arterial Elastic Membranes

Some Implications for the Pathogenesis of Arterial Diseases

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Introduction

In a previous paper attention was drawn to the presence and characters of abnormal elastic fibers in various tissues. On the basis of tinctorial reactions, fibers staining with accepted elastic methods could be separated into two apparently distinct groups, namely, "true" and "pseudo" elastic fibers (PEF's).[1-3] Arteries were among the tissues in which PEF's were encountered both in the media and in the intima.[1,2,4] In the latter location such PEF's were invariably associated with what has been called by others "reduplication" of intimal vascular elastic membranes (VEM's). It was previously shown that, in many instances, such reduplicated VEM's could not be demonstrated either with Mallory's phosphotungstic-hematoxylin (PTAH) or with others of a battery of stains. The latter finding has recently been confirmed in man by Levene,[5] while Walker[6] has also demonstrated the value of such differential staining in delineating abnormal elastic fibers in lathyrus-intoxicated rats. Levene studied what he considered to be the earliest lesions preceding coronary atheromatosis in man, reviewed much of the relevant literature, and discussed various theories as to the origin and significance of these reduplicated or longitudinally split VEM's. He concluded, ". . . the elastic lesions were thought to represent underlying foci of medial weakness. . . ."

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Moon,[7] on the other hand, has also recently described, ". . . rupture, degeneration and regeneration of the internal elastic membrane, deposition of mucopolysaccharides and proliferation of endothelial cells and fibroblasts . . . and regeneration of elastic tissue . . . as a basic mechanism of tissue reaction to injury," characterizing the early stages of arterial disease even in growing children.

It is hoped to demonstrate here that "reduplications" of arterial internal elastic membranes are almost invariably associated with the presence of one or more PEF's (defined elsewhere,[1,4] see also below), with local changes in intimal mucopolysaccharides, and usually also with increases in fibroblasts, reticulin, and even collagen. Only rarely are such "reduplicated" internal elastic membranes unassociated with the above and other perimembranous and intimal histologically and histochemically detectable changes.

In the light of modern knowledge on wound healing,[8,9] we consider these changes indicate *repair* of vascular injuries or derangements of either the *remodeling* associated with postnatal vascular growth or of *regeneration* of membranes (altered by "fair wear and tear"). Some implications of the present findings for understanding the pathogenesis of arterial diseases will here be outlined, in the hope that a new perspective may perhaps be gained on the *genesis of some arterial lesions in terms of derangements of postnatal growth, remodeling, regeneration, and repair.* Some factors, promoting or retarding the progress of such virtually ubiquitous arterial lesions to fatal

occlusive termination, will also then be considered.

Material and Methods

All of the material described in a previous publication, as well as the histological and histochemical methods there outlined,[3] were reused in the present study. In addition, however, a large number of arteries from various parts of the body of some 30 cases, ranging in age from 6 months to 104 years, were examined in detail.

All studies were conducted primarily on serially sectioned wax-embedded tissue, consecutive slides being stained by different methods, as outlined previously.[1,10,11]

Where necessary, frozen sections of formalin-fixed material were made and stained for fat (Sudan IV), also for polysaccharides (alcoholic toluidine blue; periodic acid-Schiff, and Rinehart-Abul Haj methods) and elastic fibers.

Prof. A. D. Morgan, of Westminster Medical School, made available seven specimens of wax-embedded coronary and other thrombosed arteries for study by the above methods. This series of specimens was studied during this investigation but is not here portrayed.

Observations

As indicated in detail elsewhere,[3] "true" VEM seem to have hyaline "cores," which take several stains considered by most histologists as characteristic of collagen or, at least, of collagen precursors. These stains include an orange tint with Mallory's PTAH, red or yellowish red with Van Gieson's method, red or green with the Ponceau-2R, and light green in Masson's mixture, etc. Usually, the orange-staining "core" seems to be "coated" with a material which stains a deep purple with Mallory's PTAH. As described and portrayed previously[3] in the VEM's of smaller arteries, this "purple coating" appears to have a distinctly helical arrangement around the orange "core" of the single internal VEM. A similar helical arrangement is also detectable in oblique sections of the VEM's even in larger arteries. Moreover, in particular preparations, the argyrophil fibers, around the VEM's, may lie parallel to the core (which stains red with neutral red or safranin) and may occasionally be seen "winding" around the membrane in irregular spirals. It has also been shown[3,12] that, in several experimental situations, this core and the surrounding coats in the VEM's of rats react differently, albeit simultaneously, to injury; so, too, in man. It has previously been suggested that the sleeves of reticulin and mucopolysaccharides (MPS's) constitute a built-in mechanism for the continued regeneration of the enclosed collagen-like hyaline cores of the VEM's. Rupture of the latter leads to disorganization of this regeneratory process, with repair and scar formation as a consequence.[3] Once ruptured, elastic membranes are not easily regenerated[4] and architectural derangements result. (See definitions of Regeneration and Repair, Gillman.[9])

The earliest evidence of damage to any VEM is a widening or swelling of the collagen-like core, together with fragmentation and bead-like arrangement of the material (staining purple with Mallory's PTAH) which coats this collagen-like core (Figs. 4, 6, 24, and 31). These rather slight changes may often be demonstrable even when, with elastic stains, the membrane still seems very intact (compare Figs. 3 and 5 and 13-15). However, careful study of many arteries, showing what appear to be progressively more intense changes, strongly indicates that loss of staining with elastic stains and "breaks" or defects (seen with many other methods) are simply stages which follow these initial alterations in the VEM, first demonstrable with Mallory's PTAH. A similar series of events has been described, although incompletely, in lathyrus-intoxicated rats prior to aortic rupture.[5,13]

Comparison of Figures 4, 14, 17, 23, and 29 with their respective "partners," Figures 6, 15, 19, 24, and 31, at once reveals that the one or more reduplications of the internal elastic membranes in each of these arteries, while sharply delineated with elastic stains, are *not* demonstrated at all with any of several other methods used in this study, and especially not with Mallory's

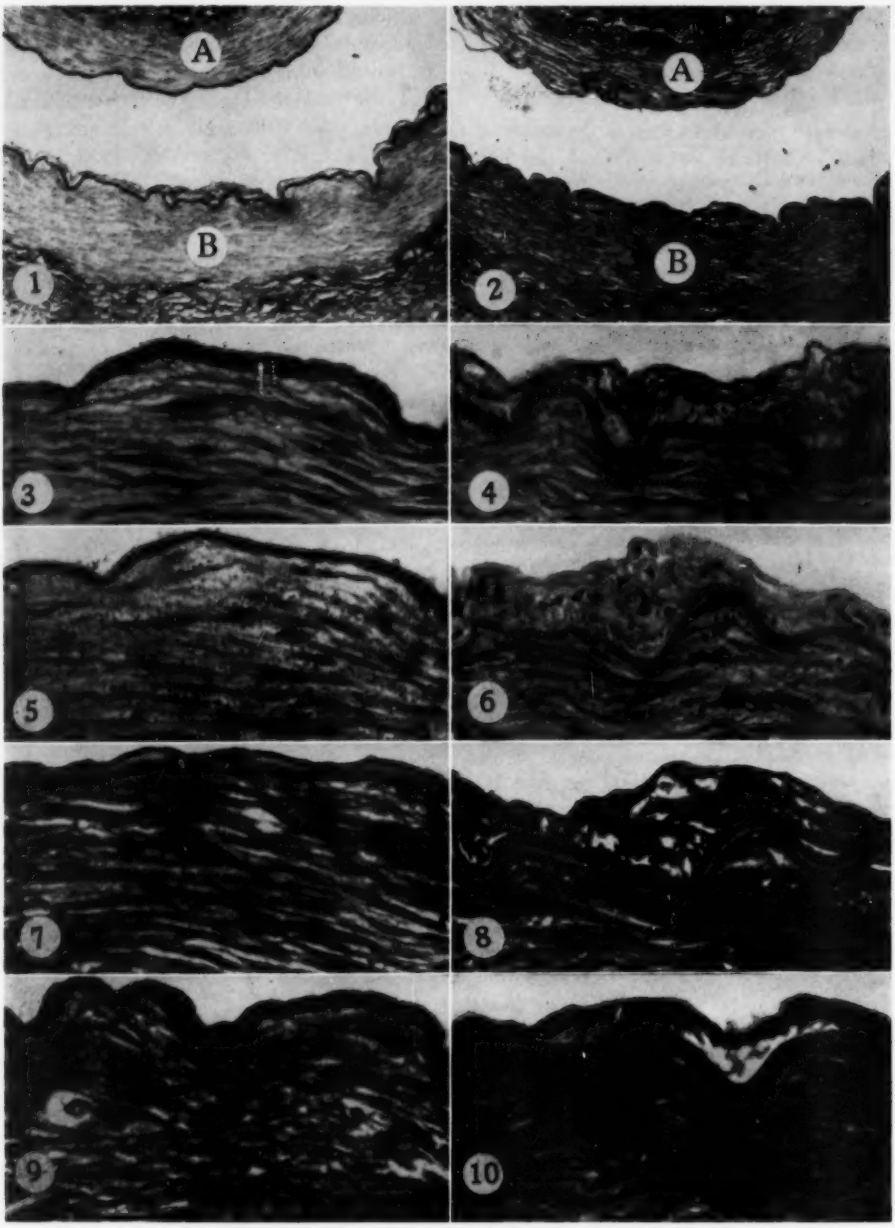

Fig. 1.—Longitudinal section of the pancreatic artery of a 14-month-old infant, to show the single internal elastic membrane superiorally (*A*) and the slightly fragmented "reduplicated" internal vascular elastic membrane (*VEM*) below (*B*). Note, too, the greater waviness of the membrane at *B*. Verhoef's method counterstained with hematoxylin; ×80.

Fig. 2.—Serial section of the same specimen shown in Figure 1, but stained for mucopolysaccharides according to the method of Rinehart-Abul Haj. Note the small amount of (dark-staining) mucopalysaccharides (*MPS*) at *A*, compared with the obviously greater amount between the innermost *VEM* and the endothelium below at *B*; ×80.

PTAH. This phenomenon, i. e., failure of reduplicated membranes (or masses of so-called "excessive elastic tissue") to stain with methods other than those used "specifically" for elastic tissue, was described in this laboratory, in 1955.[1] Identical changes have since been noted independently by Levene[5] in man, and by Walker in lathyrus-intoxicated rats.[6] However, the present study of serial sections of each of the arteries depicted here (and of many others not photographed), stained by all the methods mentioned above, discloses that *only* with "elastic" stains is more than one membrane visible (compare Figs. 16, 18, and 20; 23, 24, 26, and 27; 29, 31, 33, and 35).

Whatever the basis for the demonstrably different tinctorial reactions of single membranes, when compared with "reduplicated" membranes, these differences undoubtedly exist and will ultimately require explanation. However, even at this stage it seems justifiable at least to suggest that some chemical and/or physical differences probably exist between membranes which stain in different ways—a suggestion supported by several recent chemical and other studies.[14-16] For these reasons, therefore, those "reduplicated" membranes delineated *only* with elastic stains, but *not* by other methods, are referred to by the term previously introduced,[1] namely, "pseudoelastic fibers, or membranes" (PEF's or PEM's). Only the membranes, demonstrable with *all* of the stains here used and visible also in unstained sections by virtue of their refractility, are considered to be "true" elastic membranes.

Age Differences.—Even in infants alterations may be encountered in the morphology and staining reactions of arterial internal elastic membranes. This finding confirms those of Levene,[5] Schornagel,[17] and others quoted by them. In Figures 1 to 10 are depicted (at different powers and with different stains) the morphology and histochemistry of the internal VEM's in the opposite walls of the *same* pancreatic artery of a 14-month-old infant. Even under low power (Figs. 1 and 2) differences can easily be detected, especially in sections stained for elastic fibers or for MPS's. Thus, with elastic stains, the membrane of the lower part of the wall of this artery (Fig. 1 [*B*]) seems unduly wavy, and small irregularly spaced nonstaining zones or spots are visible along its length. The overlying intima also seems distinctly thicker and somewhat fibrillar. High-power views (Figs. 3 and 4) show the "normal" membrane (*A*, Fig. 1) to be less wavy and fairly evenly stained, whereas, at *B* not only are the unstained

Fig. 3.—High-power view of the internal *VEM* shown (*A*, in Fig. 1), demonstrating the continuous Verhoef-positive material around the membrane and the delicate elastic fibrils in the media; × 370.

Fig. 4.—High-power view of the area shown at *B* (Figs. 1 and 2), demonstrating the small interruptions in the innermost *VEM* (*B*, Figs. 1 and 2) and the fine "reduplications," with slight increase in cellularity in the overlying intima; × 370.

Fig. 5.—High-power view of a serial section to that shown in Figures 1 to 4, to demonstrate the slightly irregular outlines of the apparently intact *VEM* (*A*, Figs. 1 and 2). Mallory's phosphotungstic acid hematoxylin; × 370.

Fig. 6.—High-power view of serial section of area shown in Figure 4 (*B*, Fig. 1) indicating the "moth eaten" appearance of the blue- or purple-staining (dark) material around the innermost *VEM*. The absence of elastic-like material (pseudoelastic fibres, Fig. 4) and the moderate cellularity of this area are clearly shown. Mallory's phosphotungstic acid hematoxylin; × 370.

Fig. 7.—Serial section of the same artery shown in Figures 1 to 6, but stained for mucopolysaccharides (*MPS*). Note small amount of perimembranous (dark-staining) *MPS* around the innermost *VEM*. This perimembranous coating of *MPS* is intermittent. This field demonstrates the area shown at *A* in Figures 1, 2, 3, and 5. Rinehart-Abul Haj method; × 370.

Fig. 8.—Serial section of the same artery shown in Figures 1 to 6, showing greater amounts of *MPS* around the innermost *VEM* and in the overlying intima (*B* in the preceding figures). There is obviously an increased amount of perimembranous, as well as intimal and even medial, *MPS*. Rinehart-Abul Haj method; × 370.

Fig. 9.—Section serial to the preceding (Figs. 1 to 8), but stained for reticulin and showing normal amount of "train-line like" arrangement of perimembranous reticulin. Wilder's method; × 370.

Fig. 10.—Serial section of area *B* (Fig. 1), demonstrating the distinctly increased perimembranous and intimal reticulin. Wilder's method; × 370.

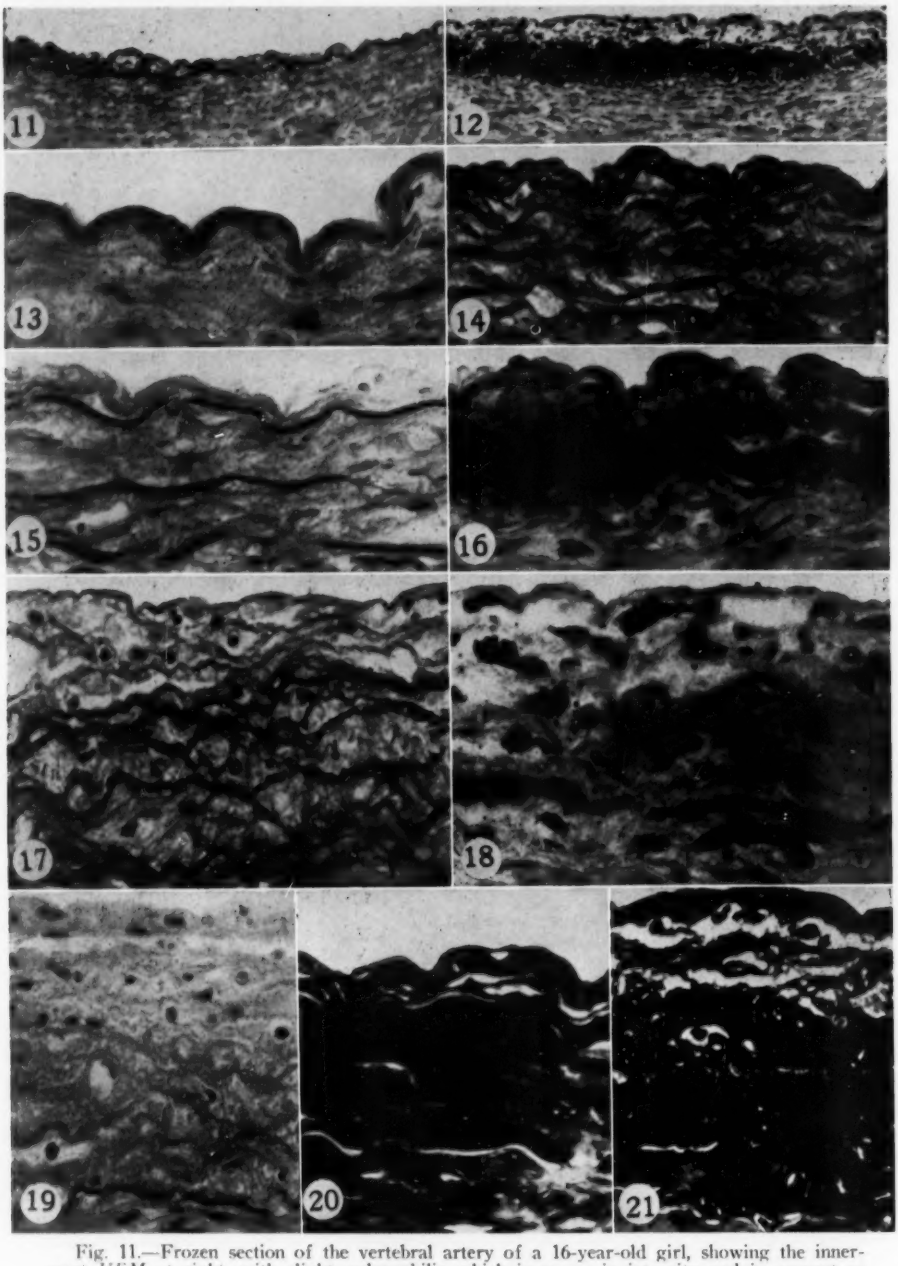

Fig. 11.—Frozen section of the vertebral artery of a 16-year-old girl, showing the innermost *VEM* at right, with slight sudanophilia which increases in intensity and in amount as one proceeds toward the (reader's) left. Where the membrane is markedly sudanophilic and thickened a considerable number of perimembranous, and especially intimal, lipid-containing phagocytes are present. Frozen section stained Sudan IV and hematoxylin; × 80.

areas on the elastic membrane clearly depicted (Fig. 4) but distinct additional Weigert-positive finer fibers are displayed in the intima, immediately below the endothelium. The low-power view of a serial section stained with Prussian blue shows distinct MPS accumulations in the subendothelial region at *B* and makes this method ideal for spotting easily the earliest deviations from "normality" (Fig. 2).

With Mallory's PTAH, the purple-staining (Fig. 5) coating, around what we have previously called the "collagen-like core" of the normal VEM (Fig. 1*A*), appears somewhat "motheaten," whereas that at *B* shows even more irregular purple staining, the orange-staining "core" being clearly displayed in the gaps within the purple-staining outer coat (Fig. 6). These irregularities, in the material which stains purple with PTAH, confirm the findings with Weigert's method, but the differences between the membrane at *A* and at *B* are perhaps displayed more strikingly with PTAH (compare Figs. 3 and 5 and 4 and 6). Moreover, accessory, or "reduplicated," membranes are not shown with PTAH, but instead the increase in subendothelial cellularity is far more obvious with PTAH than with Weigert's method.

In addition to the differences between the appearances with Mallory's PTAH and Weigert's methods, other changes are demonstrable in serial sections of the same artery stained with the Rhinehart-Abul Haj ferrocyanide technique and with Wilder's method for reticulin. With the former method a very fine, fairly regular, and often incomplete "sleeve" of ferrocyanide-positive MPS is seen around the VEM at *A* (Fig. 7); this is regarded as a normal feature of VEM's in man as well as in rats.[3] On our criteria the membrane at *B* is obviously abnormal (Fig. 8) because of

Fig. 12.—This figure depicts the continuation of the circumference of the vessel from the left edge of Figure 11 and shows the complete disappearance of the thickened sudanophilic internal *VEM*, associated with a marked increase of both extra- and intracellular lipid and a considerable cellular thickening of the overlying intima (see also Figs. 18, 19, and 21). Frozen section stained Sudan IV and hematoxylin; × 80.

Fig. 13.—High-power view of "normal" portion of the intima of the vertebral artery (at reader's right in Fig. 11), indicating the very slight amount of sudanophilic material around the intact intimal *VEM* (see also Figs. 14, 15, and 20). Technique as for Figures 11 and 12; × 370.

Fig. 14.—Wax-embedded section of the same part of the artery as that shown in Figures 7 to 13, demonstrating the thick unbroken resorcin-positive innermost *VEM*, with no overlying intimal cellularity but with some indications of "reduplication" of this innermost *VEM*. This would correspond to the area of increasing intensity of fat staining seen near center of Figure 11. Weigert's method; × 370.

Fig. 15.—Serial section to Figure 14, to show a single purple-staining inner *VEM* and no "reduplication" thereof, as well as the absence of cellularity of the overlying intima. Mallory's phosphotungstic acid-hematoxylin; × 370.

Fig. 16.—High-power view of the left-hand edge of Figure 11, showing the heavy extracellular sudanophilia around the remains of the innermost *VEM* and the increase in intimal cellularity—many of the cells here containing phagocytosed lipid. Technique as for Figures 11, 12, and 13; × 370.

Fig. 17.—High-power view of wax-embedded section of the left-hand edge of the area shown in Figure 12. The multiple reduplications of the innermost *VEM* and the thickening and cellularity of the intima are clearly shown. Weigert's elastic method; × 370.

Fig. 18.—High-power view of the middle of the portion of same frozen section as shown in Figure 12. The complete disappearance of the sudanophilic *VEM* is evident, remnants only being here visible. The increase in intimal thickness and cellularity, as well as in fat-containing phagocytes, is also striking. Frozen section stained Sudan IV and hematoxylin; × 370.

Fig. 19.—High-power view of wax section of the same part of the artery shown in Figure 18, showing the absence of purple-staining "membranes" in the thickened cellular intima (Figs. 17 and 18), the first purple-staining fragmented membrane being visible toward the bottom of this figure. Mallory's phosphotungstic acid-hematoxylin; × 370.

Fig. 20.—Serial section of the same artery shown above but stained for *MPS*. This figure shows the small amount of dark-appearing *MPS* lying above the clear "normal" part of innermost *VEM* near the top of the field (compare with Figs. 13 and 14). Rinehart-Abul Haj method; × 370.

Fig. 21.—Serial section of area shown in Figures 18 and 19, which is a portion of the artery's circumference abutting that shown in Figure 20, demonstrating the thickened cellular intima, heavily laden with mucopolysaccharide, lying above the innermost *VEM* occupying the same position (near the bottom of the field) as that demonstrated in Figure 19. Rinehart-Abul Haj method; × 370.

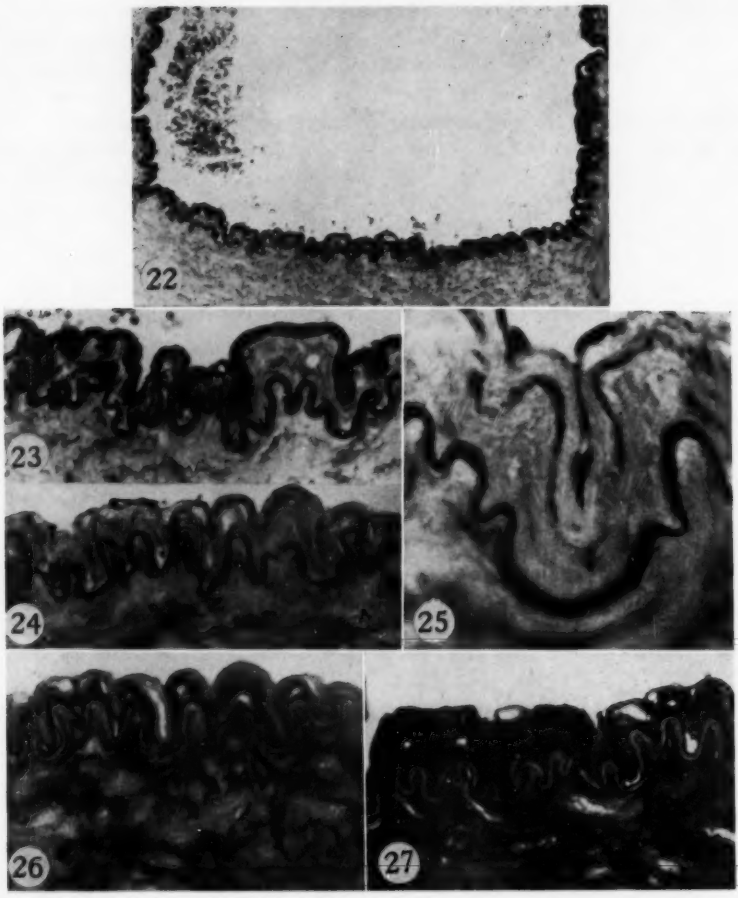

Fig. 22.—Low-power view of the radial artery of a 30-year-old man who died an acute traumatic death. The "reduplication" of the innermost *VEM* around almost the entire circumference of this vessel is clearly shown. Verhoeff's elastic stain; × 80.

Fig. 23.—High-power view of the section of the intima (enclosed in the rectangle in Fig. 22) showing the clearly "reduplicated" elastic membranes in the intima, without any increase in cellularity. Verhoeff's method; × 370.

Fig. 24.—Identical area to that shown in Figure 23, demonstrating the presence of only one purple-staining membrane, with remnants of another indicated in the overlying noncellular intima. Mallory's phosphotungstic acid-hematoxylin; × 370.

Fig. 25.—Very high-power view of a portion of Figure 24, to show the purple-staining wavy internal *VEM* and residual threads (with an arrangement suggesting a spiral) in the overlying noncellular intima; × 800.

Fig. 26.—High-power view of serial section of same area shown in Figure 24, indicating the very limited increase in reticulin fibrils in this area. Wilder's reticulin method; × 370.

Fig. 27.—Serial section to Figures 23 to 26, showing slight increase in perimembranous and intimal *MPS*. Rinehart-Abul Haj method; × 370.

(*a*) the very irregular thickening of the immediately perimembranous MPS "sleeve"; (*b*) the distinct increases in the intimal polysaccharides (already noted in Fig. 1), and (*c*) the associated slight increase in the underlying medial mucopolysaccharide. Wilder's method displays the fine "train-line"-like reticulin round the virtually normal membrane at *A* (Fig. 9), while widening of the "core," alterations in the immediately perimembranous reticulin, and distinct increases in the overlying subendothelial reticulin are shown in relation to the slightly abnormal portion of the membrane at *B* (Fig. 10).

Alterations in the VEM's, similar to those depicted and described above, have been observed often in many other arteries and at all ages. However, with ageing or with the superimposition of various pathological processes modifications in these reactions occur. Thus, in a 16-year-old girl, who died from toxemia in the third week of typhoid, a single vessel, examined with our routine battery of stains, displayed what seemed to be stages in the serial events leading to fragmentation and associated chemical alterations in a previously intact internal VEM. Similar changes were seen in several of the other arteries of this girl. The alteration in the vertebral artery, here described, exemplifies these changes. It also seems important to mention here that the following description of this girl's vertebral artery is based on observations made along different segments of the circumference of a *single* internal vascular elastic membrane (Figs. 11 to 21). This is regarded as reasonable evidence that the lesions, apparently as different as those in Figures 13 and 18, are merely stages in the progress of a single pathological change, which is more advanced in one part of the membrane and even absent from other segments of the same membrane.

The notable findings are outlined in Figures 11 to 21. The mildest (and possibly earliest) change seems to occur in, on, or around the "core" of the membrane, which swells and takes Sudan IV lightly. At this stage, the lipid is clearly located on or around the swollen membrane and not within cells (Figs. 11 and 13), a finding similar to that portrayed by Enos et al.[18] However, as the membrane seems to deteriorate its surrounding layer of lipoidal material increases both in amount and in staining intensity, and only now are fat-containing phagocytes also present around the membrane and in the overlying intima. These fat-laden cells increase in numbers as the swollen membrane becomes more sudanophilic but invisible in unstained frozen sections, owing to the loss of its refractility. Pari passu with these alterations in fat staining (seen in frozen sections) the following are notable in wax-embedded sections stained with Mallory's PTAH. The membrane loses its purple-staining outer coat while the orange-staining "core" widens, and intimal cellularity increases progressively as one moves along this membrane (Figs. 11, 12, 15, and 19). With Verhoeff's or Weigert's methods or even when stained with orcein, reduplicated membranes are obvious (compare Figs. 14 and 17) even in locations where, in serial sections, only one (? fragmented) hyaline core is detectable (Figs. 13, 14, 15, 17, and 19). This increase in intimal cellularity, intense perimembranous sudanophilia, and loss of purple staining (with PTAH) can be correlated with increased irregularity in the arrangement of the fragmented reticulin fibers, as well as with notable accumulations of ferrocyanide-positive MPS (Figs. 20 and 21); metachromatic material (with toluidine blue) is not detectable at this stage, but there does seem to be an increase in the periodic acid-Schiff (PAS)-positive substances.

In only one of my specimens was more than one membranous structure displayed below the intima with Mallory's method. This was a radial artery of a 30-year-old subject who died an acute traumatic death. In this instance elastic stains disclosed a clearly reduplicated internal VEM around the greater portion of this artery's circum-

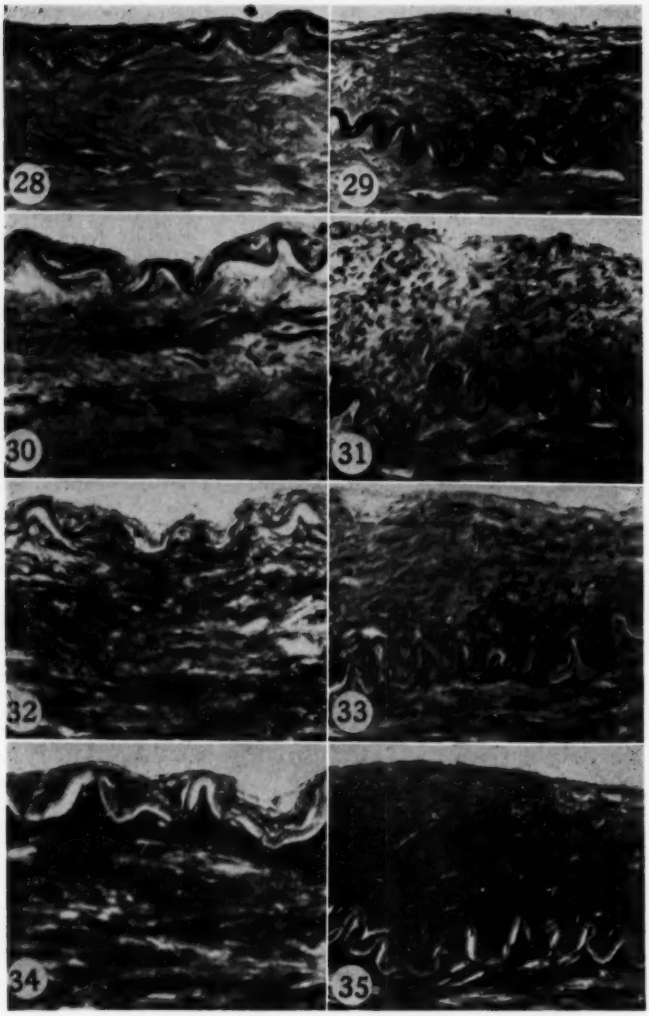

Fig. 28.—One portion of a circumference of an artery, the opposite side of the same section of which is shown in the odd numbered Figures 29 to 35. This is from a 104-year-old man. Only apparent "reduplication" of the innermost *VEM*, with suggestive increase in overlying intimal delicate elastic-like fibrils is here shown. Weigert's method; × 170.

Fig. 29.—The opposite side of the same section of the artery shown in Figure 28, showing distinct "reduplication" of the innermost *VEM*, with a considerable bed of fine elastin-like fibrils in the much thickened overlying intima. The membrane here is also much wavier than on the opposite side. Weigert's method; × 170.

Fig. 30.—Serial section of the same field in the same artery as shown in Figure 28. Note only small fragments of purple-staining material in the very slightly thickened overlying intima, which is not cellular here. The innermost *VEM* is not evenly purple-staining, and only fragments of such purple-staining material are lying around the gray-appearing orange-staining (? collagen-like) "core" of the innermost *VEM*. Mallory's phosphotungstic acid-hematoxylin; × 170.

Fig. 31.—Serial section to field shown in Figure 29 (i. e., same artery as in Figs. 27 and 30). Note single *VEM*, intermittent purple-staining of the very wavy innermost *VEM*, which has a gray zone (orange-staining, i. e., ? collagen-like) around it. The overlying intima is here shown as heavily cellular (compare Fig. 29). Mallory's phosphotungstic acid-hematoxylin; × 170.

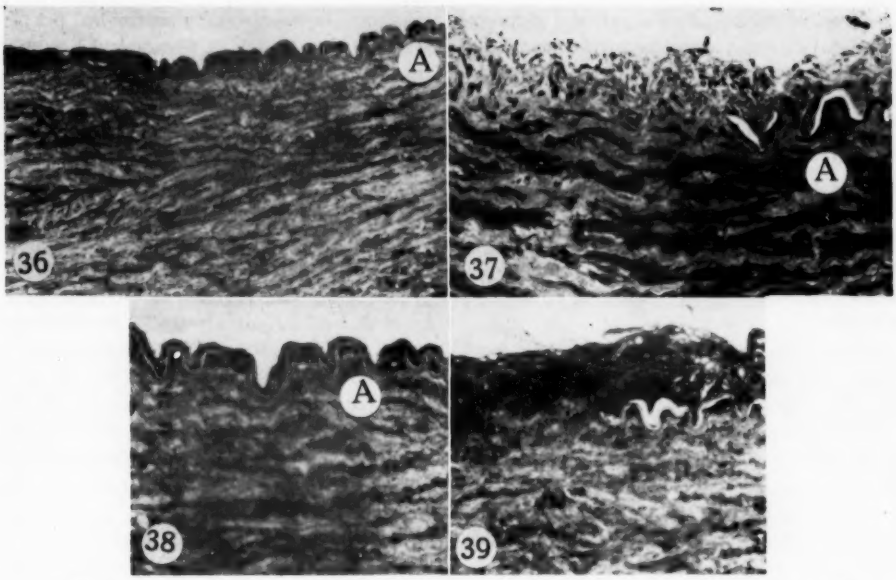

Fig. 36.—Inner portion of a branch of the popliteal artery in a patient with thrombosis of the main vessel. Note (right) clearly definable but very wavy innermost *VEM*, which disappears towards the left-hand portion of the figure. Around the right-hand portion are intermittent patches (*A*) of *MPS* accumulation, and this *MPS* is considerably more abundant at the left of the figure, where membrane is not visible at all. Rinehart-Abul Haj method; × 40.

Fig. 37.—High-power view of a portion of the artery (enclosed in the rectangle in Fig. 36) at the point where the innermost *VEM* disappears. Note the small "breaks" in the *VEM* at right and the progressive increase in intimal cellularity, proceeding from right to left, where the membrane disappears completely. Mallory's phosphotungstic acid-hematoxylin; × 170.

Fig. 38.—High-power view of a section of the vessel shown at *A* in Figure 36, showing a distinct highly wavy *VEM*, with slight increases in *MPS* intermittently along this membrane and particularly in the areas (*A*) where the membrane has "snapped." Same method as Figure 36; × 170.

Fig. 39.—High-power view of area (within rectangle in Fig. 36), showing the marked increase in perimembranous, and especially in intimal, *MPS* and disappearance of the *VEM* toward the left of the figure. This membrane is clearly degenerating; × 170.

ference (Fig. 22). As can be seen in Figures 22 and 23, the deeper (more medial) of these two membranes was somewhat thinner than the other (subintimal) one. The core of the former membrane was clearly stained orange with Mallory's PTAH, while remnants or "beads" of the purple-staining outer "coating" material were seen (Fig. 25). There was no associated increase in intimal cells, in reticulin, or in ferrocyanide-positive mucopolysaccharide (Figs. 26 and 27). This combination of reactions may perhaps, therefore, be regarded either as the earliest stage of degen-

Fig. 32.—Serial section of the same artery as shown in Figures 28 to 31, but same area as Figures 28 and 30. Note irregular reticulin around the innermost *VEM* and some increase in medial reticulin. Wilder's method; × 170.

Fig. 33.—Serial section to Figures 29 and 31, showing marked, irregular increase in intimal and in perimembranous reticulin. Only one membrane is visible here. Wilder's method; × 170.

Fig. 34.—Serial section to Figures 28 to 33, same area as Figures 28, 30, and 32. Note heavy but intermittent perimembranous dark-staining *MPS*, with small amounts of intimal and larger amounts of medial *MPS*. Rinehart-Abul Haj method; × 170.

Fig. 35.—Serial section of area shown in Figures 29, 31, and 33, demonstrating heavy but intermittent *MPS* accumulations around the innermost *VEM* and in the overlying much thickened cellular intima. Note again only one membrane detectable. Rinehart-Abul Haj; × 170.

eration or even as an entirely different process, viz., one associated with the remodeling of this membrane. The latter suggestion is favored, more especially since defects or gaps are not demonstrable in the more medial of the two membranes, the latter also being clearly displayed by other methods. Could this be an example of *true regeneration* of the VEM—especially since no evidence of *repair* (so striking in the other specimens here displayed) was detectable?

All of the features described above were seen even in single arteries but were also noted in many arteries from much older patients, several of whom had advanced degeneratory changes in other vessels. The findings, described below, are regarded as more advanced stages of the lesions noted above. Thus, the reactions in one artery of a 104-year-old man, who died of bronchopneumonia, are exemplified in Figures 28 to 35. Advanced degeneratory lesions, including extensive calcifications, were encountered in several parts of his aorta and other arteries. However, these are not germane to the present report, which is devoted essentially to the development of the early intimal lesions. The coronary arteries in this 104-year-old man were all patent and showed no large or advanced atherosclerotic plaques. Nevertheless, intimal lesions of the order displayed in Figures 28 to 35 as well as other changes (similar to those described above) were common not only in the coronaries but in many other arteries as well.

It should be noted here that the even-numbered Figures 28 to 34 represent one sector of the wall of the *same* coronary vessel, another nearby sector of which is depicted in the odd-numbered Figures 29 to 35. In the one sector, regarded as virtually normal (Figs. 28 to 34), the internal VEM stained normally with both Weigert's method and orcein, only fine fragments of additional elastic fibers being scattered in the overlying very slightly and irregularly thickened intima (Fig. 28). With Mallory's PTAH the intima was seen to be only slightly cellular, but there were very lengthy "defects" in the normally continuous purple-staining coat (black in photograph) around this VEM. In consequence, much of the orange-staining core was visible (gray in photograph, Fig. 30). The perimembranous reticulin was very little altered in this part of the VEM (Fig. 32), but there was a distinct increase in the thickness of the perimembranous "sleeve" of MPS, as compared with that customarily seen in healthy vessels of younger subjects (compare Fig. 34 with Fig. 7). In contrast, the abnormal sector of the intima of this coronary artery showed, with elastic stains, distinct reduplication of the unduly wavy internal VEM, together with many fine elastic fibers in the overlying much thickened intimal plaque (Fig. 29). Yet, as shown above, only the deeper (more medial) of the "two" membranes (Fig. 29) stained with Mallory's PTAH (Figs. 29 and 31); the more intimal membrane was not visible at all with this stain. Moreover, this single membrane (demonstrable with PTAH) showed only patchy purple staining and was surrounded by a very broad zone of orange-staining material (? collagen), comprising the "core." This is taken to imply thickening of the core itself, together with the early laying down of new perimembranous collagen. The overlying intima was highly cellular, was devoid of collagen (Fig. 31), but was rich in reticulin (Fig. 33); there was also a notable patchy thickening of the immediately perimembranous reticulin (Fig. 33). The overlying plaque was laden with ferrocyanide-positive MPS, and the perimembranous MPS sleeve of the internal VEM was also patchily thickened. (Compare Fig. 35 with Figs. 7, 20, 27, and 38.)

That the changes around the internal VEM's and the related intimal lesions described above in all probability represent stages in a degeneratory process is supported by the reactions seen in a small division of the popliteal artery, the major vessel having thrombosed after gross atheromatous changes.

The following conditions were found in one sector of the patent smaller artery

(Figs. 36 to 39): with elastic stains, the internal VEM appeared to be intact but was reduplicated; in the Rinehart-Abul Haj preparation (Figs. 36, at *A*, and 38), apart from a few tiny breaks *in its continuity* (indicated by arrows in Figs. 36 and 38), this part of the membrane seemed intact. However, there were slight increases in the perimembranous MPS, especially around the "gaps" or breaks in the membrane (Fig. 38), a phenomenon which we have described elsewhere in the early stages of aortic damage in lathyrus-intoxicated weanling rats.[13] However, in PTAH-stained sections many more "breaks" were seen even in this apparently intact part of the membrane (Fig. 37, *A*), and its purple-stained "coating" was very irregular. Whereas in Weigert preparations a single and even reduplicated membrane was seen around almost the entire circumference of this artery, sections stained by all of the other methods (applied routinely in this study) disclosed major and minor defects in the internal VEM (Figs. 36, 37, and 39). With PTAH, the sudden "cessation" of the membrane is striking (Fig. 37), as is the mild cellular overlying intima (Figs. 36, 38, and 39). The minimal amounts of MPS, present in relation to the virtually intact portion of this membrane (Fig. 38), as compared with the gross increases in the obviously disrupted parts (Fig. 39), are clearly demonstrated in Figures 36, 37, and 38, the transition being well shown in Figure 36. The possible significance of these findings will be discussed below in the light of the literature and of our own previous studies of those changes in VEM's which we associate with deranged remodeling during growth and after acute injuries.

Finally, it should be mentioned that only in the specimens in which the relatively early changes (indicative of *degeneration* of the internal VEM and associated with distinct evidence of repair—fibrosis) were moderate and patchy, were minor alterations in *medial* reticulin or MPS detected. Thus, medial lesions were *not* usually found in association with the *early* intimal regeneratory or repair processes here described but seemed to supervene only after the intimal lesions were much farther advanced.

Particularly notable in all the arteries here shown was the total absence of intimal fibrin depositions, despite the intimal lesions. As is well known, intimal fibrin deposits are invariably demonstrable most strikingly with Mallory's PTAH or with Masson's method. Yet they were not seen in any of the preparations of the arteries here portrayed. Such intimal fibrin deposits were, however, encountered in much of our material but seemed to occur in the much later stages of the pathogenesis of the intimal lesions, not presently under discussion. Such intimal fibrin deposits and the frequently associated mural thrombi can therefore probably be regarded as *secondary* to those repair processes which seem to affect the intimal VEM's first.

Comment

In his illuminating critical analysis of the pathogenesis of atherosclerosis, Duff [19] drew attention to certain points on which pathologists are apparently in general agreement and which have relevance here. For example, it is commonly taken for granted that intimal fat deposits are primary and fibrosis is secondary; intimal fibrosis probably predisposes to additional lipid accumulation; the earliest lesions are probably "fatty streaks," and these are frequent in early adult life; finally, in these fatty streaks lipid is detectable intercellularly as well as intracellularly. However, contrary to general opinion, Duff also suggests that fat accumulation may perhaps be antedated by subtle changes in the endothelium, ground substances, or other intimal components and that these preliminary changes may then predispose to localized lipid accumulations, which in turn may be further facilitated by hemodynamic factors, the state and concentration of blood lipids, etc.

There have, in fact, been several studies, in the relevant recent literature, indicating that accumulation of MPS in the intima may

precede lipid depositions, scar formation, and the progress of these lesions to atheromatous plaques with attendant complications.[7,20-22]

However, among the fundamental questions still unsatisfactorily answered are what then promotes such intimal MPS deposits, and also, why do they occur predominantly in certain arteries? For, if these MPS accumulations are indeed the primary lesions and if they, in turn, facilitate or promote subsequent lipoidosis, scarring, etc., then it is clearly important to focus attention on the origin and, if possible, on the etiology and mechanisms involved in the production of such intimal MPS accumulations. It is toward the answering of these questions that I believe the material presented above may make at least one contribution—especially when viewed in the light of our previous experimental studies on the healing of acute arterial injuries and on vascular remodeling with growth in rats.[8,9,13,23]

Regeneration and Repair of Injuries.—In previous studies we adduced evidence indicating that *single* acute injuries to vessels in rats may culminate, months later, in arterial fibrosis, contracture of the resulting scar tissue with attendant stenosis (of coronary and other smaller arteries), and later even by thrombosis.[3,8,9,23] In these previous studies, stages in the healing of acute arterial injuries were compared with those recently described by others during the repair of cutaneous wounds.[12,13,24] Attention was there drawn to the close similarities between the repair processes in these two types of connective tissues. Stress was also laid on the much longer time taken in injured arteries (as compared with skin) for scars to develop and then to undergo contracture and subsequent expansion. Some evidence, from the literature, indicating that the same holds also for human arteries, was there presented.[3,8,9,23] In this connection it also seems important to mention Poole, Sanders, and Florey's recent demonstration[25] that endothelium scraped from the aortic surface (in rabbits) does not regenerate completely, even after seven months. This supports our previous contention that vascular tissues heal far more slowly than do those of the skin; for, even extensive and deep cutaneous abrasions may regenerate completely within a few weeks.

It, therefore, seems likely that, as in experimental animals, so too in man, acute vascular injuries (*a*) may heal with scar formation and (*b*) may do so slowly and that (*c*) contracture of such vascular scar tissue may culminate only months (or in man perhaps even only years) later in localized hardening and stenosis and, with additional intimal erosions and fibrin deposits over such rigid scars, in ultimate occlusion.

We have already adduced experimental evidence that vessels are probably remodeled as they grow.[8,12,13] Such vascular growth (and associated remodeling) is perhaps greatest at the following three periods in life: (1) late in fetal life, (2) early in postnatal life, and (3) especially perhaps during that period of very active and extensive growth so typical of adolescence—both in rats and in man.[26,27] Data have also been presented elsewhere showing that vascular remodeling probably involves delicately integrated lysis and neogenesis of the membranes, analogous perhaps with that encountered during the growth and remodeling of long bones.[12,13] It has also been suggested by us, on the basis of data emerging from the study of lathyrus (odoratus) intoxication in rats, that (*a*) the more intimal VEM's are probably remodeled earliest and most frequently, and this process, when deranged, is usually associated with localized accumulations of MPS which may be followed by repair processes, with consequent intimal and even medial scarring; and (*b*) remodeling may be deranged either by (1) excessive lysis or (2) depressed synthesis of the components of the VEM's—probably the latter, at least in lathyrism.[8,13] Still further experimental data that have now become available strongly support these views.[6,9,12,13]

I believe the above facts and our interpretations thereof constitute important links in the chain of evidence which is desirable, firstly, to interpret the data here presented and, secondly, to expedite our understanding of the pathogenesis of arterial regeneration and degeneration in general.

As for the data presented above, it has here been shown that (*a*) abnormalities in VEM's may occur early in life, thus confirming the observations of others [5,17,28]; (*b*) reduplicated internal VEM's are probably not "true" elastic tissue; (*c*) when more than one membrane is displayed by so-called specific elastic stains only one is discernible with other techniques; (*d*) extracellular fat probably appears on or around injured VEM's before lipid is found within perimembranous or intimal phagocytes; (*e*) such accumulations of lipids are always associated with (and possibly preceded by) those MPS aggregations now known to be related to repair or remodeling of the affected VEM; (*f*) internal VEM's are very frequently damaged, without any associated medial lesions, and (*g*) fat and certainly MPS accumulations are frequently, if not consistently, followed by cellular and reticulin increases, associated decreases in MPS, and other evidence of scar maturation.

It also appears that many of the changes, here described, may occur either when membranes are reduplicated or, alternately, when they are lysed. Thus, the lesions described in Figures 1 to 9 and 21 to 27 were *not* associated with marked intimal cellularity or scarring, whereas those in Figures 11 to 20, 28 to 35, and 36 to 39 *did* have distinct, albeit fairly early, intimal scars—as judged from the masses of related MPS's, cells, and reticulin—all known to precede collagen synthesis.[24]

It is here tentatively suggested that the lesions characterized by membrane "reduplication" and relatively slight increases in intimal MPS, cells, and fibers, may represent minor or early disturbances in the *regeneration* or remodeling, probably going on continuously in normal VEM's—especially the most intimal ones. The other reactions, notable for the considerable associated intimal and perimembranous cellularity, actual lysis (disappearance) of portions of the membranes (Figs. 36, 37, and 39), reticulin increases, and massive MPS aggregations, are put forward as examples of vascular *repair*.

The frequent occurrence in the arteries of a centenarian of the lesions here displayed (Figs. 28 to 35) is perhaps due to a progressive deterioration of normal *regenerative* capacity, probably attendant on ageing, with consequent supervention of repair with resultant scarring. On the other hand, the lesions depicted in Figures 36 to 39 are known to have been associated with extensive and advanced disease in other closely related arteries and perhaps represent a more malignant deterioration (than in the aged), in which some underlying metabolic disturbance interfered with normal membrane regeneration. In disease, lysis of the VEM's probably proceeds more actively than the usually closely linked synthesis of the components of these membranes, i. e., dissociation of lysis from neogenesis occurs (compare reaction in lathyrism).[6,8,12,13]

Postnatal Vascular Growth and Remodeling.—Very little attention has hitherto been paid to the possible role(s) of postnatal vascular growth and remodeling, and derangements thereof, in the pathogenesis of arterial lesions. Yet, processes and relationships, similar to those shown to exist between the rate of aortic growth and the production of lesions in experimental lathyrism, also probably occur postnatally in man.[8,12] Perhaps among the most striking and illuminating of these postnatal changes are those which supervene in the uterine arteries during and after pregnancy. That these vessels increase considerably in size, especially in the latter half of pregnancy, is well known, as is also their striking postpartum involution. Even although, after several pregnancies, the uterine arteries become markedly sclerotic, they may nevertheless "loosen up" and become enlarged and actively functional again within six

months of the onset of yet another pregnancy. Our initial studies of these arteries, at term, reveal massive intimal MPS accumulations, associated with lysis, and numerous "reduplications" and apparent neogenesis of intimal VEM's, together with apparent dissolution of the overlying thickened intima itself.[29] It seems that active internal remodeling of these arteries occurs during pregnancy, and this is followed by sclerosis during the postpartum recovery period. Harkness and Moralee[30] have demonstrated dramatic changes to occur in rat uterine collagen during and after pregnancy, while Bassett[31] has recently reported profound alterations in many of the connective tissues of the ewe's genital tract during pregnancy. Such gestational changes in connective tissues generally, and particularly those in the arteries, seem to merit much closer attention than they have received hitherto. All the more so in the light of the studies and excellent reviews of the literature, by Schnitker and Bayer[32] and by Husebye and associates,[33] of the relations between dissecting aortic aneurysms and pregnancy. The latter authors concluded, "(the) time relationship (between the occurrence of dissecting aortic aneurysms and pregnancy) strongly suggests that certain metabolic changes conducive to elastic tissue degeneration take place in pregnancy." It may well be that during pregnancy alterations similar to those already seen by us in the uterine arteries also take place, albeit to a lesser extent, in many other arteries of the body. Such changes may indeed appear to be "degeneratory" when they proceed to aortic aneurysms or rupture in the genetically or metabolically hypersusceptible. On the other hand, in healthy women widespread alterations in the vascular tree, attributable to the hormonal physiology peculiar to pregnancy, may perhaps even allow for resolution of preexisting intimal lesions (in coronary and other arteries), with resulting "protection" of multiparous women against coronary lesions later in life. The possible roles of the hormonal changes in pregnancy, and perhaps of relaxin in particular,[34] in permitting and promoting widespread postnatal vascular growth, regeneration, and repair do not seem to have received the attention they merit in relation to the pathogenesis and even perhaps in the treatment of arterial diseases.

Dynamics of Vascular Degenerations.—Hitherto the most widely adopted views on the pathogenesis of arterial diseases seem to imply that these lesions result relatively suddenly and during a limited age period. There is, moreover, a tendency to regard the vascular changes in older people (50 years and more) as basically similar in origin to those so common among persons between 30 and 40 years of age. The vascular changes here described may perhaps profitably be considered as resulting from *two distinct processes* which may, and often do, occur simultaneously, namely, regeneration and repair.[9] For, *derangements in regeneration* of "fair wear and tear" breakdown of VEM's may, if acute and severe, lead to ruptures of these membranes; *repair* of such lesions and localized scar formation may then be expected. Similar scars may also perhaps result from more extensive and severe injuries to the walls of vessels due to trauma, hemodynamic factors,[18,35] and nutritional, endocrine, or metabolic disturbances.[3,8,9,20] However, slow deterioration in the regenerative processes may perhaps be anticipated in the aged, and such more gradual degeneration may account for the widespread but more slowly progressive sclerosis characterizing senile vascular changes. Varying combinations of these two processes may be anticipated at different ages in different persons (or groups of people) according to their sex, associated diseases, prevailing nutritional status, and the metabolic consequences thereof.

Vessels must grow pari passu with the over-all increase in body dimensions. Consequently, the vessels are probably actively remodeled, especially during such growth periods.[8,9,12,13] The more active the growth, the greater the need for the maintenance of those delicately regulated processes of synthesis and lysis of the existing arterial

components which must ensue and for the precise supply of materials essential during these critical periods. In such circumstances even slight disturbances might produce multiple, albeit focal, lesions. Thus, the rate of growth, as determined sexually and nutritionally, may predispose adolescents in particular to widespread arterial injuries, especially of the intima which seems to be remodeled more actively and to be subjected to greater hemodynamic stresses.[8,13]

In view of the above possibilities, it was not unduly surprising to learn that the greatest increase in the incidence of aortic intimal lesions occurs between the ages of 10 and 18.[36] The greater frequency of intimal fatty streaks in Negroes during this age period may be attributable to their poorer nutritional status.

It seems probable that *all* persons develop some vascular injuries during periods of active growth. But these initial and relatively slight derangements in vascular remodeling may, if not severe or prolonged, perhaps even resolve. If not, localized intimal injuries can be expected to progress slowly to scar formation.[8] For we have shown that scars develop very slowly in arteries, even though the same sequence of histological and histochemical events ensue as in more rapidly healing cutaneous injuries.[8,9,12] The finding of Holman and associates that intimal *scars* become common only 10 to 15 years after the appearance of intimal fatty streaking seems explicable on this basis.

However, the rate of development of such intimal scars, as well as the frequency with which they deteriorate into atheromatous plaques, may be expected to vary according to prevailing nutritional and associated metabolic conditions. There is ample evidence that such lesions develop more frequently and deteriorate more rapidly in men consuming a so-called "good" (if not excessive) Western diet relatively rich in fat. We have, moreover, indicated elsewhere that, in association with free calcium ions, some MPS can split the postprandial emulsion of chylomicrons.[37] Derangements in tissue MPS and calcium content, such as may be anticipated in sites of injury or of active VEM remodeling, may thus facilitate intramural lipid depositions, such as those shown by Anitschkow[38] to occur in the injured aortae of cholesterol-fed rabbits.

Thus, given frequent or prolonged chylomicronemia, perhaps together with minor disturbances in the sizes of the emulsion particles (as a result of quantitatively and/or qualitatively excessive fat intake), lipid accumulations may be expected to occur to a greater extent and more rapidly in sites of previous vascular injury. Derangements in the fat metabolism of intimal cells, described by Rutstein et al.,[39] may perhaps further aggravate and accelerate such lipoidal degeneration in arteries.

Sex hormones are known to affect the way in which ingested fat is metabolized as well as the endogenous metabolism of cholesterol in particular (see Oliver and Boyd,[40] Fillios,[41] Gillman and Naidoo,[42] and Gillman, Naidoo, and Hathorn[43] for reviews of relevant data). Of particular relevance, however, is the finding that a severer and more prolonged chylomicronemia can be anticipated in males than in females fed a standard fat meal.[43] Sexual differences in circulating fibrin and fibrinolysins, attendant on fat ingestion, and their possible contributions to intimal deterioration also merit consideration.[8,42,43]

Alterations in sex-hormone balance, attendant on chronic malnutrition,[8,44] may be expected to modify further the rate of deterioration of the intimal lesions which are apparently so common in virtually all adolescents. For, apart from the lower calory and especially lipid intake of poorer peoples, nutritionally induced metabolic changes and the attendant hepatic diseases, so frequently associated with a tendency to "feminization" of males,[43-45] a lower chylomicronemia, and higher fibrinolysin levels (after standard fatty meals) may all modulate the rate of deterioration of intimal arterial lesions through intimal fibrin deposits and mural thrombi.[46]

These considerations may provide a rational basis for explaining the rarity of

coronary occlusive disease among certain peoples, e. g., Japanese (Enos et al.[18,35]) and South African Negroes (Keeley and Higginson[48] and Laurie and Woods[49]) despite the ubiquitous presence of vascular pathology.[50] Thus, Enos and associates[18,35] found that American males developed advanced coronary atheroma and occlusive disease at much earlier ages than Japanese males despite the fact that lesions of the coronary vessels were nevertheless present in the latter. Similar observations have been made in the Union of South Africa.[48]

It may also be possible that repeated pregnancy may have a "protective" effect on arteries (on the basis outlined above) and may modify both vascular connective tissues and endogenous cholesterol metabolism by virtue of the actions of various gestational hormones, including relaxin.[34,50] Meticulous study of this aspect of the problem may yet disclose means for attacking, even in the male, lesions hitherto regarded as irreversible.

Conclusions.—There is thus a considerable body of evidence indicating the occurrence of intimal mucopolysaccharides, cellularity, and membrane reduplications even in the arteries of young persons. These commonly encountered changes have not yet been rationally accounted for. The data presented here, interpreted in the light of our previous experimental findings in rats, may well provide a new understanding of the origin of these early lesions as well as of their deterioration into scars (with or without atheromatous changes) and ultimately with intimal fibrin deposits to occlusion by mural thrombi at an early age. Our studies of several aspects of vascular structure in health, during ageing, and in disease and of some sexually determined differences in fat metabolism, plasma fibrin, and fibrinolysin (and their responses at fat intake) may also assist in understanding the pathogenesis of these diseases.

It seems reasonable too, on the basis of all of the above considerations, to suggest that the etiology and pathogenesis of fatal vascular degeneratory disease is likely to be elucidated more rapidly if due consideration is given, firstly, to the role of postnatal vascular remodeling, regeneration, and repair in initiating intimal vascular lesions and, secondly, to sexually, nutritionally, and endocrinologically determined modifications of metabolism which may inhibit or expedite the deterioration of these early lesions and the supervention of terminal occlusive episodes at an early age. It seems unlikely that any *single* factor will be found to account for the origin and progress of these diseases and for the differing incidence according to sex, race, and economic status. Senile sclerosis, on the other hand, is attributed to a progressive deterioration of normal regeneration into repair with scar formation as a consequence.

Summary and Conclusions

Some microscopic changes, associated with very early intimal lesions and especially reduplications of vascular elastic membranes (VEM's), are described in human arteries at different ages.

Evidence is presented, suggesting that the majority of reduplicated VEM's are not "true" but rather pseudoelastic fibers, the latter defined on morphological and tinctorial criteria. Such reduplications are regarded as indicative of the remodeling of arterial intimal structures during growth or regeneration of fair-wear-and-tear changes.

Regeneration, remodeling, and repair of VEM's is associated with mucopolysaccharide accumulation, either around affected VEM's and/or in the intima, with or without increased cellularity and lipids and the formation of new reticulin and/or collagen fibers.

The possible significance is outlined of postnatal vascular growth, remodeling, regeneration, and repair in relation to the genesis, time of onset, and rate of progress of intimal lesions to occlusive endarterial disease. The possible importance of hormonal regulation of bodily growth (and therefore also of vascular growth) and remodeling, of fat intake and metabolism,

and of local changes in tissues in facilitating deterioration of the early intimal lesions is also outlined. Particular attention is directed to hormonally regulated arterial changes in pregnancy.

It is suggested that a distinction may be desirable between the pathogenesis of arterial lesions characteristic of the fourth and fifth decades of life and senile sclerosis, the latter perhaps being due in large measure to a slow deterioration, with ageing, of the normal regenerative processes into repair with consequent gradual vascular fibrosis.

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Immunohistochemical Detection of Antibody in Cell-Transfer Studies

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Cell-transfer techniques in which appropriately stimulated lymphoid cells capable of an antibody response are transferred to immunologically inert homologous recipients have been used extensively to study antibody formation. Recently it has been shown that lymph node cells (90% lymphocytes) obtained from a previously sensitized donor rabbit are capable of transferring to x-irradiated recipient rabbits the ability to make a typical secondary antibody response on exposure to the specific antigen.[1,2] In these studies, previously immunized lymph node cells no longer in the process of active antibody formation were injected subcutaneously into recipients which had been given 400 r whole-body radiation to eliminate their own ability to make antibody and which had had no previous exposure to the specific antigen.

Morphologic studies of the subcutaneous cell-transfer sites in these recipients showed that preplasma and then mature plasma cells replaced the injected lymphocytes during the antibody response.[3] Immunochemical observations provided convincing evidence that the transferred lymphocytes made the antibody,[1,2] and on the basis of serial morphologic study the lymphocytes also appeared to be the source of the developing preplasma and plasma cells.[3] However, definitive proof of antibody formation by the developing plasma cells in the transfer sites was lacking. The present studies were then undertaken to provide such evidence. The immunohistochemical technique of Coons was used to visualize the appearance of antibodies to the specific antigen and to correlate the morphologic development in the subcutaneous cell-transfer site following antigenic stimulation with the appearance and localization of antibody.

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Department of Pathology, University of Pittsbugh School of Medicine; United States Public Health Service Medical Student Trainee under Grant No. CRT-5019 (Mr. Neal).

Materials and Methods

Complete descriptions of the materials and methods used in these cell transfer studies have been published previously.[1] Donor rabbits, weighing approximately 2.5 kg., were immunized by repeated subcutaneous and intravenous injections of bovine serum albumin (BSA) totaling 230 mg. over a period of one month. Approximately 20 days after the last antigen injection, the donors were killed, and the lymph node cells from the mesenteric and popliteal lymph nodes were stripped of their investing fat, minced, and prepared as a free cell suspension. The lymph node cells were approximately 90% lymphocytes, 8% macrophages, reticuloendothelial cells, and 2% plasma cells. A minimum of 90% of these cells at the time of injection did not stain with trypan blue and thus were considered to be viable cells.

Two days before injection of the cells, the recipient rabbits, none of which had had previous exposure to BSA, were x-irradiated (400 r whole-body irradiation) to suppress completely any primary immune response to BSA for at least two weeks.[5] About 500,000,000 viable cells suspended in a solution of PVP and glucose (Macrose) were injected subcutaneously and intramuscularly over the anterior abdominal wall of the recipient rabbit. Immediately after the injection of the cells, 1.5 mg. of I^{131} trace-labeled BSA was injected intravenously into the recipient. The radioactively labeled antigen was employed for ease in later antigen determinations. The immune

response was measured by observing elimination of labeled antigen and maximum concentration of circulating antibody.[6] At various times during the response, recipients were killed and tissue from the injection sites was fixed in formalin for permanent hematoxylin and eosin (H & E) sections and also quick frozen to provide material for the Coons' fluorescent-antibody technique.[4] The morphology of the cell transfer site was also studied from connective tissue spreads or smears made from the area of the transfer site and stained with May-Gruenwald Giemsa stain.

The procedures for the preparation of specific antiserum used in the Coons' "fluorescent-antibody technique" were similar to those described in a previous report from this laboratory.[7] Sections, 5μ-7μ in thickness, were cut from the quick-frozen tissue blocks in a refrigerated cabinet (—20 C) by use of a rotary Minot microtome. Slides with the mounted tissue were immersed in absolute alcohol (ethanol)-ether (50:50) for 10 minutes. Then, they were transferred to 95% ethanol at 37 C for 20 minutes. After this, they were washed in buffered saline for 5 minutes (two changes). A few drops of BSA solution (containing 500μg. P per milliliter) was placed over the mounted tissue and allowed to stand for 30 minutes. The unbound antigen was then removed by washing in buffered saline for 10 minutes (four changes). The slides were then ready for immediate staining with the fluorescent anti-BSA conjugate. After a 30-minute exposure to the fluorescent conjugates, the slides were again washed in buffered saline for 10 minutes (4 changes). The section was then covered with a clean coverslip and stored at 5 C until microscopic observation.

The tissue was then observed for fluorescence by use of a Zeiss-Winkel microscope with a dark field condenser and a high-pressure mercury lamp as an ultraviolet light source.[7,8]

Control studies for the specificity of staining for anti-BSA were performed routinely according to the following staining scheme:

1. One section was stained with H & E.
2. One section was stained with nonfluorescent antigen followed by fluorescent antiserum, thus detecting anti-BSA.
3. One section was stained just with the conjugated antiserum, thus acting as a control for (2).
4. One section was stained for presence of rabbit γ-globulin.
5. One section was stained for presence of rabbit albumin.
6. One section was left unstained for detection of autofluorescence.

Results

The Table summarizes the observations on 36 animals killed during a period of from 4 hours to 26 days after antigenic stimulation of the transferred cells. The complete elimination of circulating antigen was observed four to six days after transfer and was indicative of a good secondary response of the transferred cells (Col. 3). The fifth column of the Table characterizes the cell population of the transfer site as observed on the H & E permanent sections, the connective tissue spreads, and also the sections studied with the fluorescent-antibody technique. Column 6 indicates the relative number of cells in the section staining specifically for antibody.

Antibody Response to Serum Protein Antigens by Lymph Node Cells Transferred to Adult Recipients

Day of Death	Recipients, No.	Day of Antigen Elimin. † 4	5	6	Average μg. Ab N/ml. Serum #	Type of Cells in Injection Site: Lymphocytes	Transitional & Preplasma Cells	Plasma Cells	Antibody Fluorescence
1	1	--	--	--	--	++++	0	0	0
2	2	--	--	--	--	+++	+	±	0
3	3	--	--	--	--	++	++	+	+
4	2	1/2	--	--	--	+	+++	++	+++
5	2	1/2	2/2	--	--	±	+++	+++	++++
6	2	--	1/2	2/2	--		++	+++	+++
8	9	--	8/9	9/9	3.55		++	+++	++
9	4	--	1/4	4/4	2.55		+	++	+
14	8	--	7/8	8/8	3.20			±	±
26	3	--	2/3	3/3	3.33				0

* Each recipient received 5×10⁶ donor lymph node cells subcutaneously and intramuscularly and 1.5 mg. I*BSA intravenously.
† Numerator is number of animals having eliminated antigen from circulation, and denominator is total number of animals in group.
# μg. Ab N obtained by multiplying μg. antigen N precipitated by 1 ml. serum at 80% antigen precipitated × the average antibody N-antigen N ratio at this point (5.5). Bleedings made 3-4 days after elimination of circulating antigen.

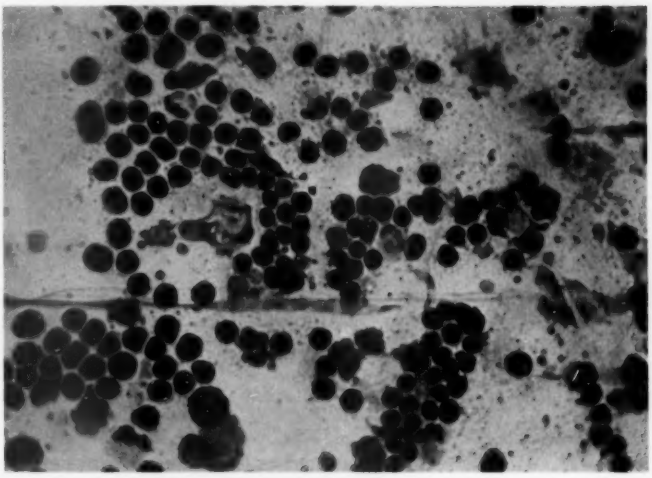

Fig. 1.—A connective tissue spread taken from a cell-transfer site four hours after antigenic stimulation. Note the numerous lymphocytes, with an occasional large immature appearing cell; × 380.

Figure 1 is a connective tissue smear stained with Giemsa stain obtained from a cell-transfer site of an animal killed four hours after antigenic stimulation. The cell smear is similar to the preinjection smear of lymph node suspension in that the cells are predominantly lymphocytes, with an occasional primitive cell plus a few cells of the host mesenchyme. Between Days 1 and 3 the number of lymphocytes decreased, and occasional large immature-appearing cells compatible with "Fragraeus' transitional cells" as well as smaller "preplasma" cells appeared, especially around nerves and blood vessels.[9] Figure 2 shows a connective tissue spread taken from a cell transfer site on the third day after antigenic stimulation. There are typical mature lymphocytes; however, an increasing number of "preplasma" cells and an occasional mature plasma cell can be seen. Antibody within cells in the injection site was first detected by use of the Coons' "fluorescent-antibody technique" on the third day, as shown in Figures 3 and 4. Most of the antibody-containing cells observed on the third day had an eccentric accumulation of antibody-containing cytoplasm and were

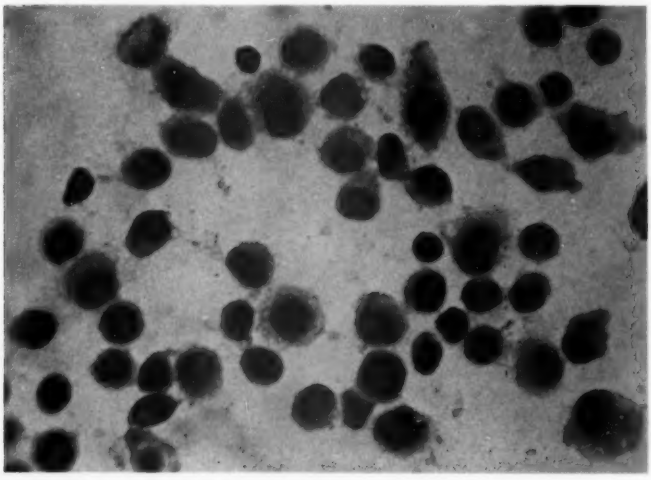

Fig. 2.—A connective tissue smear taken three days after antigenic stimulation. Numerous preplasma cells and an occasional plasma cell are seen. Note the reduction in lymphocytes as compared to Figure 1; × 600.

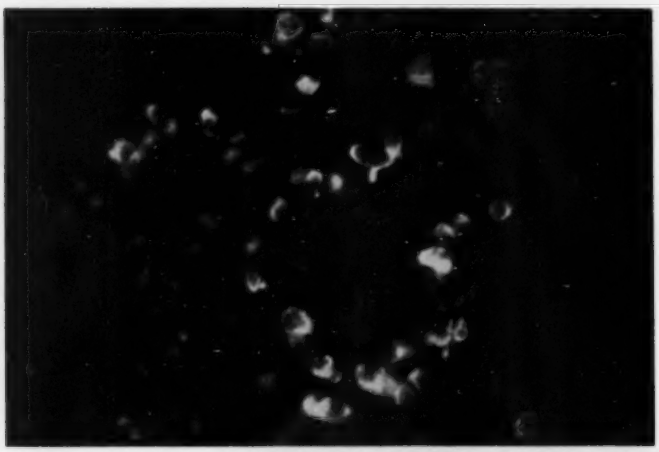

Fig. 3.—Frozen section of cell transfer on the third day stained for anti-BSA with use of Coons' fluorescent-antibody technique. The positive cells are located around a medium-sized blood vessel; reduced 5% from mag. × 300.

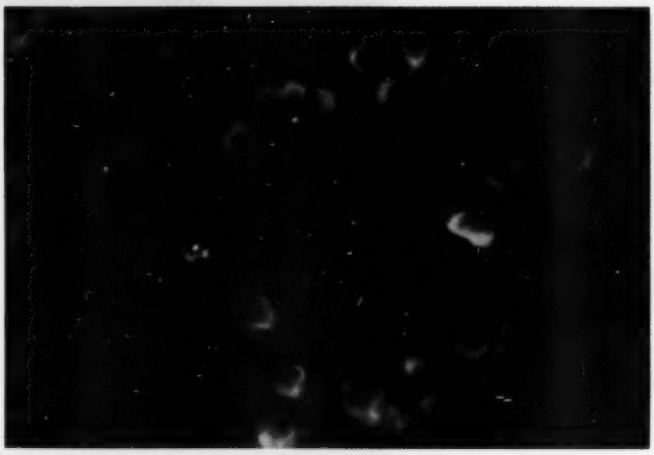

Fig. 4.—Higher magnification of Figure 3. Note the morphologic similarity between the cells containing antibody and the preplasma cells as seen in Figure 2; reduced 5% from mag. × 480.

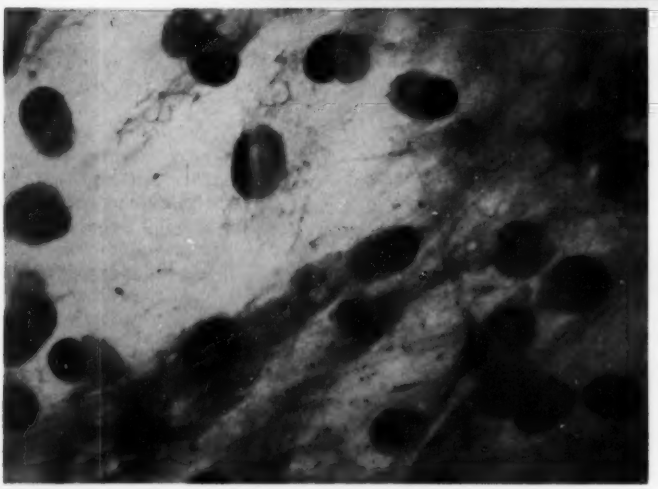

Fig. 5.—Connective tissue spread of a cell-transfer site on the fifth day. Note the increased number of mature plasma cells as compared to the third day and also the absence of mitotic activity; × 600.

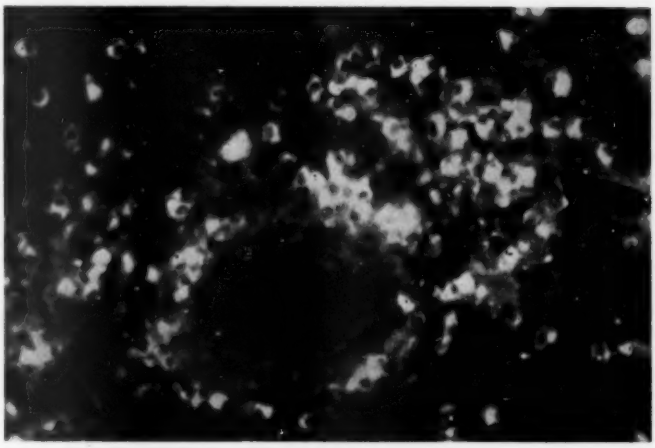

Fig. 6. — Frozen-tissue section of cell-transfer site on the fifth day stained for anti-BSA. Note the increased number of positive cells around a medium-sized blood vessel as compared to the third day (Fig. 3); reduced 5% from mag. × 192.

morphologically similar to "preplasma" cells as described by Fragraeus.[9] The number of positive-staining mature plasma cells was minimal on the third day. The fluorescence was confined to the cytoplasm of these cells and did not appear within the nucleus or in the extracellular spaces. The intensity of cytoplasmic fluorescence was found to vary widely. The more immature-appearing cells had the least intense cytoplasmic fluorescence, and the mature plasma cell had the most intense cytoplasmic fluorescence.

The maximum number of antibody-containing cells was found on the fifth day after antigenic stimulation (Figures 6 and 7). A connective tissue smear taken from the cell-transfer area on the fifth day shows a marked predominance of the "preplasma" and plasma cells (Fig. 5). Few lymphocytes are seen on the fifth day in contrast to the third day, at which time numerous lymphocytes were identified. No mitotic divisions were found at any time on the many Giemsa-stained connective tissue spreads and smears; however, an occasional binucleated cell was seen.

The maximum level of circulating antibody was found to occur on the ninth day after the cell transfer. At this time, the number of antibody-containing cells was reduced as compared to the fifth day. Now virtually all of the remaining antibody-

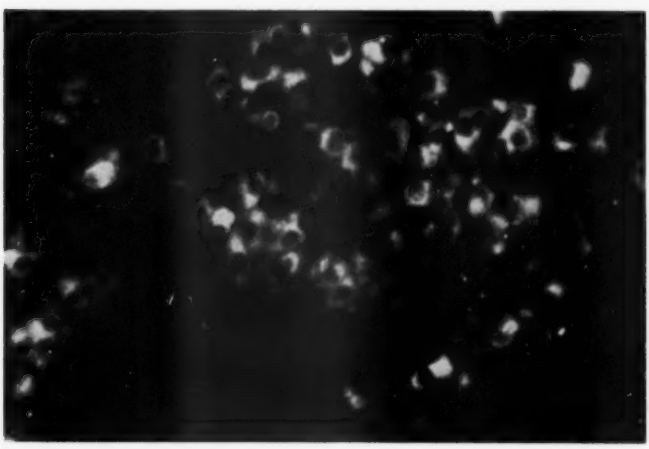

Fig. 7.—Higher magnification of the same area seen in Figure 6. Numerous typical preplasma and plasma cells are seen which contain antibody. Note that the mature plasma cells have the most intense fluorescence; reduced 5% from mag. × 300.

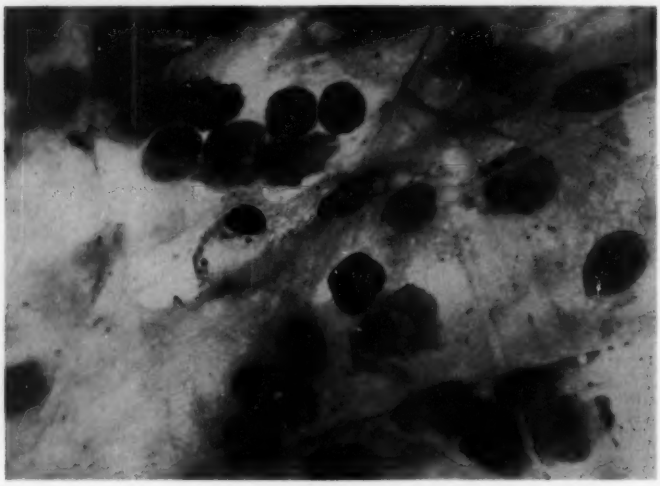

Fig. 8.—A connective tissue spread of a cell-transfer site on the ninth day. Virtually all cells are mature plasma cells; × 600.

containing cells were mature plasma cells (Fig. 8).

By the 14th day the number of antibody-containing cells had decreased markedly. At this time occasional positive cells were identified in only three of eight recipients studied. Animals were last investigated on the 26th day, at which time no antibody-containing cells were noted.

Comment

Previous immunologic studies of the secondary response to protein antigens following transfer of lymph node cells indicated that the transferred cells made antibody.[1,2] Related morphologic studies showed the development of preplasma and later plasma cells in the sites of transfer of the lymph node cells. Serial morphologic observations of the transfer sites plus control experiments transferring killed or unstimulated cells indicated that the plasma cells most likely developed from the transferred cells and not the recipient tissues.[3] These two sets of observations suggested without definitive proof that the transferred cells were simultaneously forming antibody and developing into plasma cells. In the present study it has been possible to observe the appearance of specific antibody within the preplasma and plasma cells developing in the cell-transfer sites, thereby affording substantial support for the above postulation.

Connective tissue smears taken from cell transfer sites four hours after antigenic stimulation consisted chiefly of mature lymphocytes. In the next few days, during the rise in antibody synthesis, smears from the transfer sites showed a decrease in the number of lymphocytes and an increase in the number of "transitional, preplasma, and plasma cells." Fragraeus[9] and Roberts et al.,[3] among others, found the preplasma cell to be the prominent cell during the early and most active phase of antibody production in a secondary immunologic response. Antibody within cells in our injection sites was first detected on the third day, and these positive cells were morphologically similar to transitional and preplasma cells (Figs. 3 and 4). The maximum number of antibody-containing cells was found on the fifth and sixth days. At this time there was a marked predominance of preplasma and plasma cells in the transfer sites. By the ninth day, the number of antibody-containing cells was greatly reduced. At this time all of the remaining antibody-containing cells were mature plasma cells. This supports Fragraeus' thesis[9] that the early forms of the plasma cell predominate during the most active phase of antibody production, with the mature plasma cell

appearing later. Whether the disappearance of antibody-containing cells in the transfer sites after the fifth or sixth day merely reflected the decline in antibody formation naturally occurring at this time in a secondary response,[10] with persistence of the transferred cells in a functionless state, or whether, in addition, the cells were being destroyed by a homograft reaction of the host cannot be definitely determined from the present data. However, since the disappearance of the antibody-containing cells occurred at the usual time of a homograft reaction and since either the delay of initial antigen injection for several days after cell transfer or the second injection of antigen two weeks or more after cell transfer fails to elicit an antibody response,[11] it seems likely that the host was reacting against the cells.

No mitotic activity and only an occasional binucleate plasma cell were observed during the first five days after transfer on both Giemsa-stained connective tissue spreads or smears and permanent H & E sections of the injection sites. Coons et al.[4] observed considerable mitotic activity in the plasma-cell series during the process of antibody formation in the lymph nodes of the intact rabbit. While our observations are not directly comparable with those of Coons, they do show that in this experimental milieu there can be morphologic transition of the transferred cells without significant mitotic activity associated with the synthesis of antibody. Similarly, less mitotic activity was noted by Fragraeus[9] in tissue cultures producing antibody than in the same tissue of intact animals producing antibody.

Summary

Since in these transfers of lymph node cells the time and magnitude of the antibody response and the immunochemical characteristics of the antibody in the recipient indicate that the transferred cells make the antibody [1,2] and since the antibody can be localized within developing plasma cells in the cell transfer site, it would appear likely that the plasma cells are derived from the transferred lymph node cells. Considering the minimal mitotic activity in the transfer sites it would appear that during their antibody response the transferred lymphocytes metamorphose to the plasma cells without division via the stages described.

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Congenital Polycystic Disease of the Liver

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Introduction

The true incidence of congenital polycystic disease of the liver is perhaps not as great as that generally reported. Feldman [8] reports an autopsy incidence of 0.53% and cites Ackman and Rhea, who reported an autopsy incidence of 0.16%. However, only 29 cases are authenticated from the Surgical Service of The Mayo Clinic from 1907 to 1954, inclusive, according to a recent report by Henson [9] and associates. The occurrence of one case in 3,362 autopsies and one case in approximately 4,782 laparotomies in the past 15 years at the Veterans Administration Hospital, Portland, Ore., represents autopsy and surgical incidence respectively of 0.03% and 0.02% and, although statistically perhaps not significant, infers a lower general incidence.

Since "cystic disease of the liver" and "nonparasitic cystic disease of the liver" are broad inclusive terms, it becomes desirable to set more specific criteria for definition and nomenclature of this disease.

We subscribe to the term "congenital polycystic disease of the liver" for that disease wherein there are multiple widespread fluid-filled cysts disseminated throughout the liver parenchyma and wherein such cysts are of presumed embryologic defect origin (although this aspect of the disease is still not conclusively established). This definition must exclude parasitic cysts, degenerative cysts arising from other pathological processes, "acquired" cysts from inflammatory processes, neoplasms, and post-traumatic cysts.

Submitted for publication Oct. 21, 1958.

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Report of Cases

CASE 1.—A 66-year-old white man was admitted to the Veterans Administration Hospital on July 26, 1951, because of a progressive enlargement of the abdomen of three months' duration, fatigue and dyspnea of one month's duration, and easy bruising. In 1943 he was hospitalized in another city because of fatigue and breathlessness and was told he had cirrhosis of the liver. In October, 1949, he developed severe epistaxis as a sequela of an upper respiratory infection and was hospitalized at a local hospital. Here it was noted that his liver was enlarged. Laboratory findings at that time were as follows: red cell count 3,400,000; hemoglobin 8.14 gm.; white cell count 1,200, with 58% lymphocytes; platelet count 226,000; prothrombin count 58%; total protein 5.08 gm. %; albumin 3.0 gm. %; globulin 2.08 gm. %; icteric index 4; thymol turbidity 3.5 units; alkaline phosphatase 5.9 Bodansky units; cholesterol 98 mg. %. A sulfobromophthalein (Bromsulphalein) test on one occasion revealed 2% retention and on another, 16.6% retention in 45 minutes. The serology was negative. A bone marrow study showed "a large number of normoblasts, many target cells, and a few myelocytic cells beyond the myelocyte stage; conclusion: suppression of myelocytic series." Roentgenogram showed a pneumonic infiltration in the right lower lobe. On Nov. 28, 1949, a laparotomy was performed and the diagnosis of polycystic liver disease was made.

He remained relatively well until April 14, 1950, when he again experienced severe epistaxis and also complained of pain in the hips and thighs. He was admitted to the Portland Veterans Administration Hospital on this date and was treated for the epistaxis, a prostatic abscess, and a cellulitis of the thigh. The laboratory findings on this admission were as follows: red cell count 2,640,000; hemoglobin 9 gm.; hematocrit 31; white cell count 1,450, with differential count of 70% neutrophils and 30% lymphocytes; prothrombin time 52%; sedimentation rate 61 mm. per hour; platelets 88,000; serum proteins 6.6 gm. %; albumin 3.0 gm. %; globulin 3.6 gm. %. The remainder of the liver-function tests were normal. He remained fairly well until about one year later, when he

became aware of increasing abdominal enlargement, fatigue, dyspnea, and ecchymoses of the extremities. He was readmitted to this hospital on July 26, 1951.

He was a bartender and cabinet-maker by occupation and stated he drank moderately every night.

Physical examination disclosed an obese, ruddy-faced and plethoric white man who had an extremely large abdomen and appeared in moderate respiratory distress. His temperature was 100.4 F; pulse, 82; respirations, 20; weight, 232 lb., and blood pressure, 150/82. There were a few small spider angiomata on the face and arms, ecchymoses on the extremities, and dilated veins over the abdomen. The chest revealed scattered rales in both lung bases. The abdomen was filled in the upper portion by a huge liver, which extended from the anterior superior iliac spine on the right upward into the left hypochondrium. The margin was blunt and irregular; the surface, somewhat nodular. The liver silhouette was visible on respiration. No ascites or peripheral edema were noted.

Laboratory Findings

Red blood cell count 1,450,000; hemoglobin 5.5 gm.; hematocrit 18; sedimentation rate 78 mm.; blood indices, MCV 126, MCH 36, MCHC 29; serology negative; urinalysis negative. Liver-function tests were normal except for a 20% sulfobromophthalein retention in 45 minutes. The blood smear showed moderate poikilocytosis and macrocytosis; reticulocyte count ranged from 0.5%-0.9%. Repeated blood cell counts showed a persistent granulocytic leukopenia and anemia. Roentgenogram of the chest on admission showed increased vascular markings throughout and some clouding of the right lower lung field, presumably due to pulmonary edema. Another film, on Aug. 16, showed a mottled density in the lower one-third of the right lung field. Intravenous cholecystogram and urograms were normal.

Clinical Progress

After admission the prothrombin level dropped progressively to 48%, but it returned to normal following vitamin K administration. The patient had a low-grade fever most of the time, with several spikes up to 102 F. He was considered to be in mild cardiac failure and received mercurial diuretics, with marked improvement including diuresis and rapid weight loss. The anemia failed to respond to cyanocobalamin (vitamin B_{12}), and he received whole-blood transfusions. Abdominal paracentesis in two different sites failed to yield ascitic fluid. He was digitalized and received large doses of vitamin B complex, ascorbic acid, and vitamin K. His general condition improved until Aug. 15, 1951, when he experienced the sudden onset of acute right chest pain and dyspnea. He died on Aug. 18, 1951.

Fig. 1.—Gross photograph of cut surface of liver showing polycystic disease.

Postmortem Findings

The body was that of an obese, well-developed man who presented a markedly protruberant abdomen, scattered ecchymoses, bronzing of the exposed parts of the skin, and a healed right paramedian abdominal scar. The liver was increased to a weight of 4,800 gm. and extended 12 cm. below the right subcostal margin, 10 cm. below the xiphoid process and 8 cm. below the left subcostal margin. The outer and inner surfaces of both lobes showed diffuse cystic disease (Fig. 1). The cysts varied from 2 mm. to 4.5 cm. in diameter and generally contained a thin yellow fluid but occasionally a clear or green fluid. Some cysts were loculated, but generally they were single. The remaining hepatic parenchyma appeared smooth and pale yellow-tan in the embalmed state. The spleen was enlarged to a weight of 1,300 gm. The capsule was partially hyalinized; the cut surface was congested. The esophagus was free of varices. The kidneys each weighed 230 gm. and contained multiple small cysts. The right contained eight cortical cysts each approximately 8 mm. in diameter and one larger cyst 2.2 cm. in diameter. The small cysts contained a relatively clear fluid, while the larger one contained a coagulated dark brown material. There were no cysts in the pancreas. The lungs were heavy, the right weighing 1,100 gm. and the left 775 gm. The right lower lobe contained a hemorrhagic infarct with associated fibrinous pleuritis and an embolus. Both lungs showed considerable edema. The brain was relatively normal.

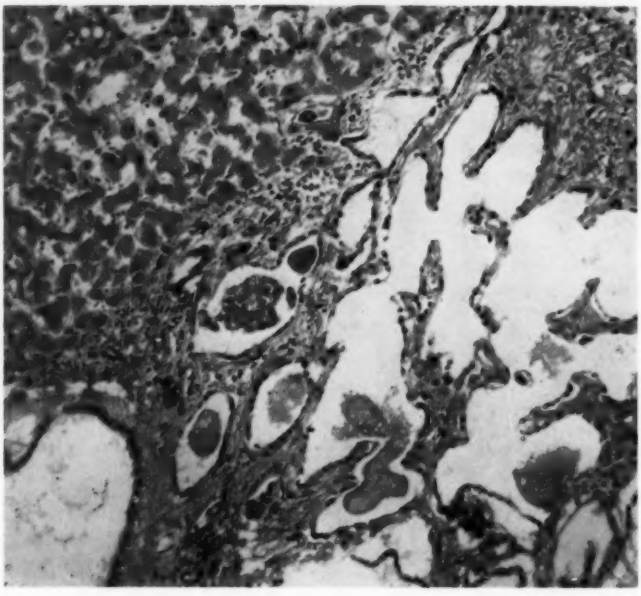

Fig. 2. — Microscopic section of liver showing cysts with characteristic cuboidal epithelial lining. Hematoxylin and eosin; × 115.

Microscopic study of the liver revealed single and confluent cysts generally lined by a single layer of cuboidal or low columnar bile-duct-like epithelium but occasionally only by collagenous tissue (Fig. 2). The cysts often contained a homogenous acellular material. The hepatic cells in general were well preserved, although a few showed fatty metamorphosis. Some portal triads contained an excess number of lymphocytes in association with a few plasma cells and granulocytes. There was no cirrhosis. The largest renal cyst contained cholesterol clefts and calcareous debris and was lined by connective tissue. The lung sections confirmed the gross findings. The heart showed moderate coronary sclerosis and mild myofibrosis. There was also an incidental finding of a papillary adenocarcinoma of the thyroid.

CASE 2.—A 61-year-old white man entered the Veterans Administration Hospital, Portland, Ore., on May 23, 1958, complaining of constant aching abdominal pain without radiation. This pain started gradually four months prior to admission and slowly but progressively increased. It was associated with meals but had no relationship with bowel function, activity, or position. There was no history of nausea, vomiting, jaundice, or gastrointestinal hemorrhage. He had had 15 lb. weight loss. He had no urinary symptoms except for mild nocturia.

The past history was unremarkable except for chronic rheumatoid arthritis and a perforated duodenal ulcer in 1947, for which he underwent laparotomy and closure.

Physical examination disclosed a well-developed, well-nourished 61-year-old white man, ambulatory and in no distress. The blood pressure was 130/80; temperature, 98.6 F, and pulse, 76. The abdominal examination disclosed a well-healed right-rectus surgical scar. There was no distention or evidence of intraperitoneal fluid. Bowel tones and abdominal percussion were normal. The kidneys and spleen were nonpalpable, but the liver edge was palpable 6 cm. below the right costal margin and appeared slightly blunted but regular. Immediately beneath the liver in the area of the gallbladder there was an acutely tender round poorly delineated mass which moved slightly with respiration but did not appear to be attached to the liver. The rectal examination was normal.

Laboratory Findings

The hemogram and urogram were normal. The serology was nonreactive. Liver-function studies disclosed a negative cephalin flocculation, 1+ thymol turbidity, 6.4 King-Armstrong units of alkaline phosphatase, and 0% sulfobromophthalein retention in 45 minutes. Roentgenograms of the chest were normal. Upper gastrointestinal films showed a duodenal cap deformity consistent with

Fig. 3.—Gross photograph of polycystic liver taken in surgery.

a chronic peptic ulcer. The barium enema was normal but for depression of the hepatic flexure, as though by a round extrinsic mass. The oral cholecystogram showed adequate gallbladder function and a crescentic indentation on the lateral margin near the infundibulum suggested an imposing extrinsic mass. The intravenous urogram disclosed prompt concentration of the contrast agent, with mild blunting and irregularity of the right renal collecting system, the significance of which could not be determined.

Operation

On June 10, 1958, the abdomen was entered through the previous right-rectus muscle-splitting incision. The surface of all lobes of the liver was studded with tense translucent cysts up to 2 cm. in diameter. The intervening liver parenchyma appeared entirely normal. Beneath the right lobe and in the proximity of the gallbladder there were several tense cysts, the largest of which was approximately 10 cm. in diameter (Fig. 3). All visualized cysts contained clear fluid except one of the largest, which contained gray-brown turbid fluid. All cysts were attached to the liver, and no free floating cysts were visualized. The kidneys were normal in size and shape, except that their surfaces were studded with multiple small readily palpable cysts. There was no splenomegaly.

Treatment consisted of aspiration of the largest cysts and biopsy of the wall of several of them, with careful closure of all cysts entered.

The postoperative course was uneventful, and the patient tolerated oral intake well on the second postoperative day. He was discharged on the eighth postoperative day, feeling well and relieved of his symptoms at the time of hospital admission.

Pathology

The biopsy specimens disclosed a cyst wall lined with low to normal cuboidal epithelium with a thin subjacent layer of hyalinized connective tissue. The adjacent liver parenchyma was entirely normal histologically. The embedded aspirated fluid from one cyst disclosed only clear fluid with a few granulocytes and a rare lymphocyte; that from an adjacent cyst disclosed brown cloudy fluid with total protein of 5.9 gm. %, total bilirubin of 1.4 mg. %, cholesterol of 68 mg. %, and cholesterol esters of 16 mg. %.

Comment

The first case was characterized by marked hepatomegaly and moderate splenomegaly. The patient was anemic and showed a hypoprothrombinemia and granulocytic leukopenia. The marrow hypoplasia was attributed to chronic infection. He showed symptoms of early hepatic failure. Some laboratory findings indicating liver dysfunction included a 20% sulfobromophthalein retention, a hypoprothrombinemia, and slightly altered serum proteins. During his last admission he developed a macrocytic anemia, presumably on the basis of liver disease. Other cases in which hepatic failure developed have been reported by Niemetz [11] and associates.

The second case clearly illustrates the typical findings portrayed by Comfort [4] and associates in their very complete description of 24 cases at the Mayo Clinic. The patient was in apparent excellent health, and he had no manifest evidence of far-advanced hepatic disease. His symptoms were referable to the upper abdomen, and none pertained to the urinary tract. The progress of the disease was slow but persistent, and there were no acute episodes of pain, nor were there any symptoms suggesting a biliary tract inflammatory process.

The appearance of the liver at laparotomy showed diffuse and extensive involvement with perhaps 50% of the hepatic parenchyma replaced with cysts, all in the absence of any remarkable over-all hepatomegaly. The larger cysts beneath the liver were attached to the liver, but some by only a small pedicle. It is presumed that their significantly greater size was due to their superficial location, thereby allowing more uninhibited expansion.

Pathogenesis.—The typical widespread involvement; the slow progression of the disease; the association with other anomalies,[3] notably the renal cysts, and the reports of familial incidence [9] all point strongly to a congenital origin of polycystic disease of the liver. To establish this as a distinct disease entity one must exclude cases of solitary or a few isolated cysts, granted that focal areas of anomalous duct development may lead to cystic change. It seems more likely, however, that such cysts should arise from inspissated secretions or postinflammatory duct obstruction.

In a very complete and comprehensive analysis of the probable pathogenesis of both intrahepatic and extrahepatic bile duct anomalies, Moolten,[10] in 1943, described the two roles of embryologic cell function: that of cell differentiation to form highly specialized cells and that of elaboration of substances from primordial cells that induce other cells to undergo differentiation into specialized structures. An aberration in one or both of these functions early in biliary tree development as the hepatic diverticulum develops from the midgut might well account for the extrahepatic anomalies (e. g., biliary duct atresia); an aberration in later development may account for intrahepatic atresias or ectopic ducts with multicystic change. The unique case reported by Clagett and Hawkins,[3] in 1946, may present an intermediate situation. In this case there was extensive left lobe involvement with an evidently normal right lobe. It is likely that the embryologic dysfunction occurred just as the left hepatic duct was developing and after its budding from the common hepatic duct, thereby carrying the anomaly through the entire left biliary tract. Further evidence suggesting a congenital origin is that the disease has been described in newborn infants.[9]

Diagnosis and Treatment.—There are no pathognomonic signs or symptoms of congenital polycystic disease of the liver, but prolonged progressively increasing symptoms referable to the upper abdomen in a patient seemingly in good health should suggest the possibility of this disease. Henson [9] and associates noted that 76% of 29 patients had a palpable mass and that in 21% polycytic disease of the liver was an unsuspected finding at laparotomy for another disease. In an analysis of 24 cases, Comfort [4] and associates noted symptoms referable to the liver in only 10 (42%). In occasional instances liver-function studies and especially the sulfobromophthalein test are helpful. In one of Niemetze's cases the sulfobromophthalein showed a retention of 29% after 45 minutes and the patient showed functional impairment of the liver. In the absence of any consistent physical or laboratory findings the definitive diagnosis then rests with direct visualization of the liver and, if necessary, biopsy. Two cases were diagnosed at peritoneoscopy by Niemetz [11] and associates, and in one of these a needle biopsy of the liver was performed. This biopsy showed a liver cyst lined by cuboidal epithelium and a few normal liver cells. In one of the case reports [2] of the Massachusetts General Hospital

needle biopsy proved the diagnosis of polycystic liver disease.

There is no truly satisfactory treatment. Aspiration of the cysts at laparotomy gives temporary relief of symptoms. Resectional surgery is rarely feasible. Fortunately the over-all outlook for the patient is good, as the disease is only very slowly progressive and death is ordinarily due to other causes. Henson [9] reported one patient with a 31-year follow-up and seven patients with more than 10 years' follow-up. Rarely are there complications other than discomfort. However, Deutsch [6] reported a case complicated by ascending cholangitis with multiple liver abscesses and subsequent death. Those patients with associated renal cystic disease suffer renal complications much more commonly, hence preservation of renal function is the critical problem with them.

Summary and Conclusions

Two documented cases, one autopsy and one surgical, of congenital polycystic disease of the liver are reported, with the pathology and clinical features of each described.

It is recommended that this disease be defined and classified as a distinct entity, with differentiation from other hepatic cystic disease.

Multiple widely disseminated cysts with otherwise normal hepatic parenchyma, a slow progressive course with a reasonably good prognosis, chronic upper abdominal pain, and infrequent complications characterize the disease.

Unless death from other causes intervenes, death is ordinarily due to hepatic failure from progressive displacement of hepatic parenchyma.

Since there are no pathognomonic findings, the definitive diagnosis is made only by direct visualization of the liver and, if necessary, biopsy. Needle biopsy has occasionally established or substantiated the diagnosis.

Liver impairment is occasionally reflected by altered liver-function tests.

Dean Altman assisted in the preparation of the illustrations and photomicrograph.

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Electron Microscopic Observations on Capillaries of Atherosclerotic Aorta

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Although capillaries have been demonstrated repeatedly in the tunica intima of atheromatous arteries,[1-6] their significance has been variably assessed. By producing recurrent small hemorrhages in the arterial intima, they may have a bearing on the genesis and progression of the atheromatous change,[2,6-8] or by their rupture in the terminal stages of the sclerotic process they may produce sufficient subendothelial hemorrhage to occlude a coronary artery.[1,2,9] The idea has also been expressed that capillaries have little significance in the pathogenesis of the disease except in the organization of mural thrombi.[4,5]

Because the resolution provided by electron microscopic methods makes possible the positive identification of capillaries and of interstitially located erythrocytes, this work is presented in confirmation of certain of the light-microscopic findings cited above.

Materials and Methods

The aorta on which the present observations are based was one of a series whose fine structure is presently being studied. Human autopsy material is usually unsuitable for electron microscopic study because of postmortem changes. Fortunately the tissue in the present case was reasonably well preserved, although it was obtained about 30 minutes after death. The subject was a man of 72 years who died from bronchogenic carcinoma with widespread metastases.

A piece of tissue, consisting of the whole thickness of the vessel wall and measuring about 2×1.5 cm., was removed from the middle region of the descending thoracic aorta. It was immediately immersed, intact, into 50 ml. of 1% osmium tetroxide buffered to pH 7.3 and fixed for 90 minutes at room temperature.[10] The entire piece was then transferred to 70% ethanol, in which it was stored overnight. After this period of fixation and partial dehydration thin shavings were cut from the intimal surface. These shavings were cut into strips to produce blocks for embedding about 2×1×0.5 mm. It is emphasized that the aortic tissue was not cut into blocks in the unfixed state, and so there was no possibility of producing a capillary hemorrhage by trauma. The small blocks were quickly dehydrated in graded ethanol and embedded in a mixture of butyl and methyl methacrylate (80:20) containing 0.5% benzoyl peroxide and allowed to polymerize in ultraviolet light. Thin sections were examined with a Philips EM100A electron microscope at 60 kv. Sections for light microscopy were stained by Altmann's method for mitochondria. This method has been found useful for osmium-fixed tissue, which is refractory to staining with hematoxylin and eosin.

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From the Department of Microscopic Anatomy, University of Western Ontario Faculty of Medicine.

Observations

Grossly, the intimal surface of the piece of aorta selected showed confluent white fibrous plaques over most of its area, but it had also a raised part of pale yellow color. There was no evidence, from the naked-eye appearance, of hemorrhage, ulceration, or thrombosis.

A number of blocks of intimal tissue showed the usual electron-microscopic appearance of human atherosclerotic lesions, the details of which are outside the scope of this paper but may be summarized as follows: an intact single layer of endothelial cells; in the subendothelial tissue, some vacuolated macrophages, some smooth muscle cells and some cells resembling fibroblasts but not definitely identified as such, and between the cells great numbers of collagen fibrils and granular amorphous material.

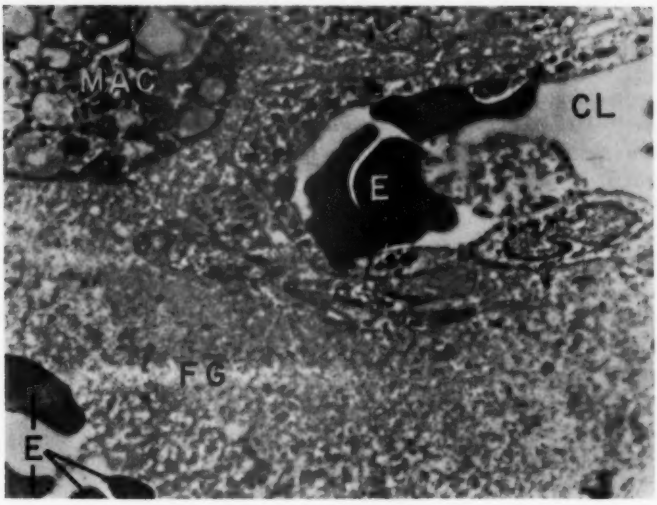

Fig. 1.—Part of a capillary is seen in the aortic intima 60μ from the lumen. The capillary contains erythrocytes (*E*) in its lumen (*CL*) and is lined by flat endothelial cells. The dark bodies in the cytoplasm of these cells are mitochondria. The capillary is surrounded by the granular and fibrous extracellular material (*FG*) of the plaque and close to it are part of the cytoplasm of a macrophage (*MAC*) and some extravasated erythrocytes (*E*). Reduced 25% from mag. × 6,000.

One block showed the additional features of vascularization by capillaries and interstitial hemorrhage.

Two capillaries were observed at a level of 60μ to 70μ from the lumen. They consisted of flattened endothelial cells (Fig. 1) and contained erythrocytes in the lumen. They appeared to differ in no essential way from capillaries in other locations.[11] A definite basement membrane could not be visualized, perhaps because fixation was not adequate. The capillaries were surrounded by granular and fibrous material of the plaque. In this material were found vacuolated macrophages and extravasated erythrocytes.

Some of the macrophages contained homogeneous inclusions of circular or oval profile with an electron density the same as that of adjacent erythrocytes (Fig. 2). Occasionally an erythrocyte, or a part of one, was situated against the plasma membrane of the macrophage.

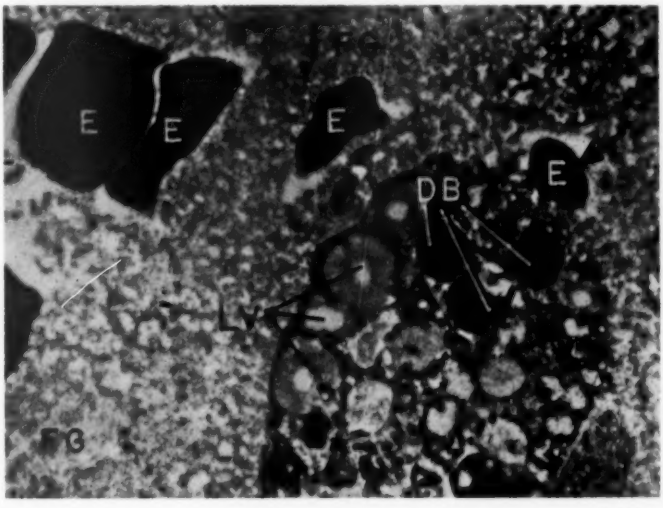

Fig. 2.—Another part of the same macrophage is shown in relation to sections of several erythrocytes (*E*), all in an extravascular location. One erythrocyte (arrow) is situated in close relation to the macrophage. Contained in the cytoplasm of the macrophage are a number of lipid vacuoles (*LV*) of light and medium density and also three dense bodies (*DB*), showing the density of erythrocyte cytoplasm. Reduced 25% from mag. × 8,000.

The nature of the inclusions in macrophages, as seen with the electron microscope, can only be a matter for speculation at the present time, as specific cytochemical tests have not yet been developed. However, on the basis of the comparison of electron- and light-microscopic observations, the majority of the vacuoles of low and intermediate density are thought to be lipid inclusions. On the basis of the comparability of both density and homogeneity between some of the dense bodies and the erythrocyte cytoplasm, the conclusion was reached that the very dense ones were parts of erythrocytes. The striking resemblance which they bear to erythrocytes outside the macrophages suggests to me that they are parts of extravasated red blood cells which have been taken into the macrophages by phagocytosis.

Capillaries could be recognized with the light microscope with much less certainty than in the electron micrographs. In sections cut from the same methacrylate block and stained by Altmann's method (Fig. 3) they appear as double rows of elongated nuclei. Extravasated red blood cells and macrophages are also seen.

Comment

The observations presented here have no bearing on the etiology of atherosclerosis, since the disease in this case was well advanced. Further study with the electron microscope of early fatty streaks might help to assess the conclusions of Paterson's group, namely, that vascularization occurs early in the atherosclerotic process and repeated small hemorrhages lead to fatty change.

However, the present observations do provide some information on the structure of capillaries in the later stages of atherosclerosis and also confirm the existence of interstitial hemorrhages in the intima.

Crawford and Levene [5] have called the vascular channels in arterial intima "abnormal capillaries," and obviously in arising directly from a large artery [1-6] and in having a high intraluminal pressure, such capillaries are abnormal. At a distance of only 60μ from the aortic lumen one might expect the pressure transmitted to the tissue in which the capillaries lie to be in the range of the arterial blood pressure and to follow closely the systolic and diastolic levels. A capillary in this environment could only remain patent if its intraluminal pressure were of the same order. The *transmural* pressure in the capillary would be low—perhaps in the normal range. Thus it is perhaps not surprising that these capillaries, even when

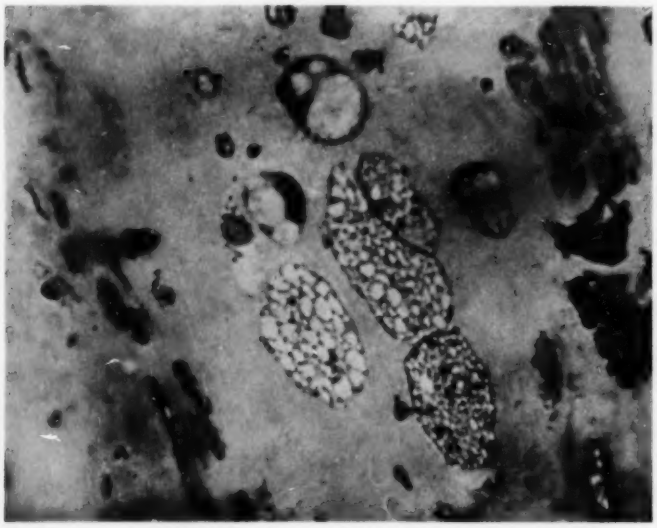

Fig. 3.—A section 1μ to 2μ thick from the same methacrylate block as Figures 1 and 2, showing a capillary, some vacuolated macrophages (foam cells), and a few extravasated erythrocytes. The capillary is indicated by a double row of elongated nuclei running diagonally in the lower left quadrant. Altmann's method; reduced 15% from mag. × 1,500.

examined by the electron microscope, show no significant morphologic abnormality. They do not have or require any special structure to cope with their high intraluminal pressure since their transmural pressure is not high.

Although Drury [9] found capillary hemorrhage in the intima of a proportion of cases of coronary occlusion, he expressed the opinion that hemorrhage in the intima was more often the result of rupture of surface endothelium and penetration of blood into the wall through unlined channels. This explanation of intimal hemorrhage does not fit the present case, since, in all blocks studied, the endothelial layer was intact.

From the relatively normal appearance of the extravasated erythrocytes it would appear that the hemorrhage reported in this case was of recent origin. In spite of this fact, the phenomenon which was interpreted as the uptake of fragments of erythrocytes into macrophages had already started to manifest itself. My experience with the examination of a number of aortas fixed for various periods of time in osmium tetroxide convinces me that these dense inclusions differed from osmiophilic lipid inclusions in their homogeneity, their sharp margins, and the absence of shrinkage artifacts. It seems probable that such cells, at a later stage, contained the iron which Paterson and his colleagues [8] have been able to demonstrate as a diffuse coloration with the Prussian blue test.

Summary

Capillaries have been visualized by electron-microscopic methods in the intima of an atheromatous human aorta. They have the usual structure of capillaries in other sites. Extravasation of red blood cells and their incorporation into macrophages were observed.

Mr. William Daniels and Mr. Charles Jarvis provided technical assistance, and Dr. J. C. Paterson, advice. The Atkinson Foundation provided a grant for the purchase of the electron microscope.

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Simple Cerebral Scars

Intrinsic Cortical Circulation and Its Relation to Focal Vascular Lesions

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The occurrence in the cerebral cortex of scars presumed to be a residual effect of vascular disease is well known to pathologists. This type of scar is usually the result of obstruction of one or more of the smaller cortical arteries (ischemia) or some other cause (anoxemia) of lessened blood supply to the cortical structures. Such scars are very distinct from the simple cortical defects resulting from contusions on one hand and the complex fibroastral scars consequent to compound comminuted fractures of the cranial vault on the other. This group of vascular scars in a sense seem to bridge the gap between these two types of traumatic lesions. In many instances the causative circulatory disorder or vascular disease is very evident; in others the disturbance in blood supply is indirect and often obscure. For the present, however, I am not so much concerned with either the primary or ultimate cause as I am with the structure and mechanism of formation of the scar itself. Particular attention will be paid to the local vascular reactions leading to what appears to be a proliferation of blood vessels and the elaboration of reticulin in the formation of the residual lesion.

In this study a new method for the demonstration of the finer cortical vasculature has been utilized (Courville).[1] It is an adaptation of the Laidlaw silver technique for the demonstration of reticulin. By clearing sections 250μ thick it becomes possible to visualize the vascular network of the cortex in three dimensions. This method gives one an entirely different concept of scars which originate in vascular occlusion.

As a preliminary to a study of the scars themselves, a brief review will be made of the pattern of intrinsic cortical vasculature.

Intrinsic Cortical Vascular Pattern

Every student of diseases of the nervous system is well aware of the necessity of an intact vascular pattern in maintaining the integrity of the cerebral cortex. Yet remarkably little attention has been paid to this system as a whole in describing structural alterations of the cortex. One is usually content with fragmentary glimpses of the arterioles, capillaries, and venules as chance observations on thin sections of these vessels. Because the subject of cerebral cortical scars cannot be separated (as will presently be shown) from the cortical vasculature itself, a brief review of this subject is first in order.

Current knowledge of the human vascular pattern has usually depended upon the benzidine stain or some modification of it (Pickworth).[2] This method demonstrates the red blood cells within their lumens rather than the walls of the vessels. Hence, one's impression of the size and arrangements of the finer cortical vessels is gained largely from their contours. This secondary impression is often imperfect and largely negative, particularly if the brain has been removed from an embalmed body. A modifi-

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From the Cajal Laboratory of Neuropathology, Los Angeles County Hospital, and the Division of Nervous Diseases (Neurology) College of Medical Evangelists.

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cation of the Laidlaw technique[3] was developed by Miss Catherine Mahoney, research technician of the Cajal Laboratory. This new method has made it possible to demonstrate the different cortical vessels in rather thick sections cleared of their nuclear and cellular detail so as to see them in three dimensions.

The cortex is found to be supplied with blood through short arterioles which enter the cortical gray matter at right angles and by branches of longer arterioles which penetrate the underlying white matter. These vessels of fairly uniform caliber give off branches which are progressively reduced in diameter (precapillaries) until they actually become capillaries. The capillaries themselves are small curving thin-walled channels which, after communicating with two to four other capillaries, terminate in an afferent (prevenule) of a cortical vein. These veins are characterized by their richer arborization, their short thick afferents, and their larger, irregular, thin-walled trunks. The capillary network which intervenes between the arterioles and venules is characterized by numerous loops of uniform diameter which

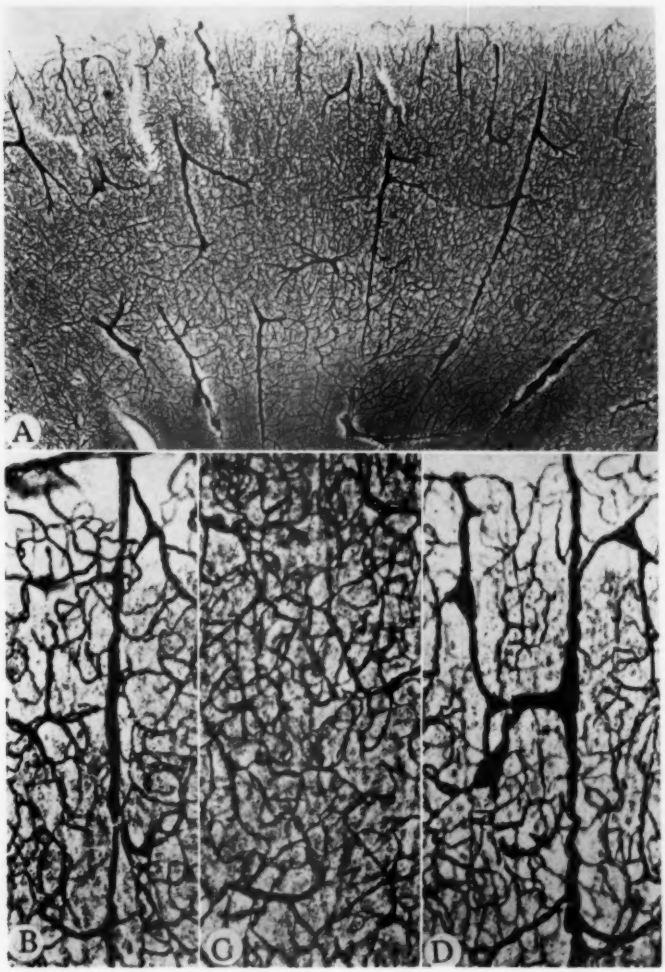


Fig. 1.—Normal vascular pattern of the cerebral cortex. *A*, low-power view, showing general arrangement of vascular pattern; reduced 10% from mag. ×22. *B*, cortical arteriole with fairly uniform diameter and simple branching, reduced 10% from mag. ×80. *C*, arrangement of the capillary pattern; reduced 10% from mag. ×80. *D*, cortical venule, showing irregular contour and expansions where afferents join the main trunk; reduced 10% from mag. ×80. Mahoney silver preparations.

occupy the cortex in its three dimensions. These details are shown in Figure 1.

Brief Review of Literature on Vascular Scars

When pathology was coming into its own as a specialized science well over a century ago, the not infrequent occurrence of vascular scars of the brain was readily recognized. In his atlas, Cruveilhier [4] depicted nodular atrophy of the cerebral and cerebellar cortex. The association of such lesions with advanced degenerative changes in the arteries of the brain seemed obvious. It was about half a century later, however, before special attention was paid to the histologic structure of these scars and their similarity to the cortical atrophies of early life designated as microgyria or lobar sclerosis (Jendrassik and Marie)[5] was noted. This detail of the problem has been stressed in a contemporary study (Courville)[6] and need not be discussed further in this connection.

More to the point was the observation of Spatz,[7] who associated localized irregular cortical atrophy in the brain of elderly persons with chronic diseases of the arteries. These cortical lesions, however, had already been given critical microscopic attention by such capable neuropathologists as Spielmeyer [8] and Jakob.[9] The present contribution is limited to the specific role that the cortical blood vessels play in the production of these scars.

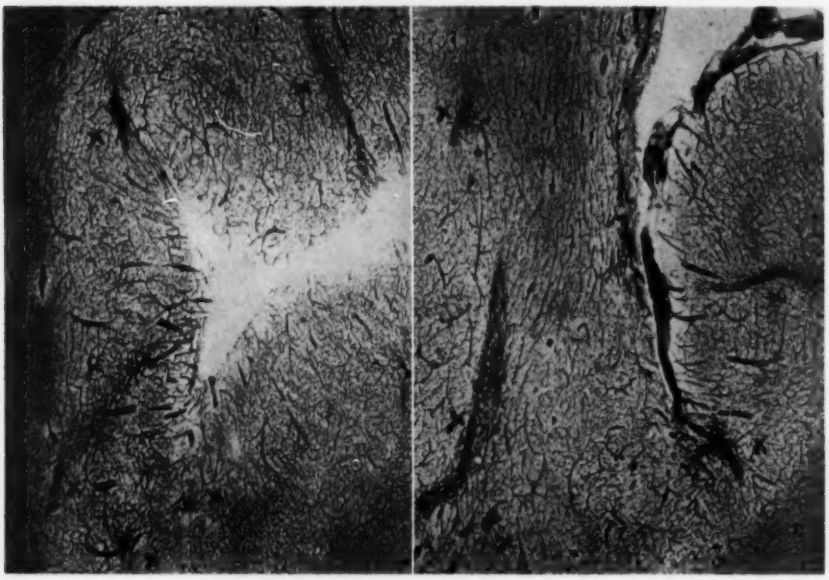
Material Studied

For the past year, selected cases of vascular lesions of the cerebral and cerebellar cortex have been subject to study, as well as examples of old vascular lesions of the basal ganglia. Since the lesions in only 12 cases have been evaluated by this means, this study at best can only be considered as introductory.

Classification of Vascular Scars of the Encephalic Gray Matter

It soon became evident by this survey of the series of histological preparations available for examination that the usual concepts of vascular scars of the gray matter of the brain have been too circumscribed. Instead

Fig. 2.—Multiplicity of vascular scars in cerebral cortex. Multiple focal scars in cerebral cortex (*x*), showing different types found within a small area. These scars would not be visible to the naked eye; × 18. Mahoney preparation.



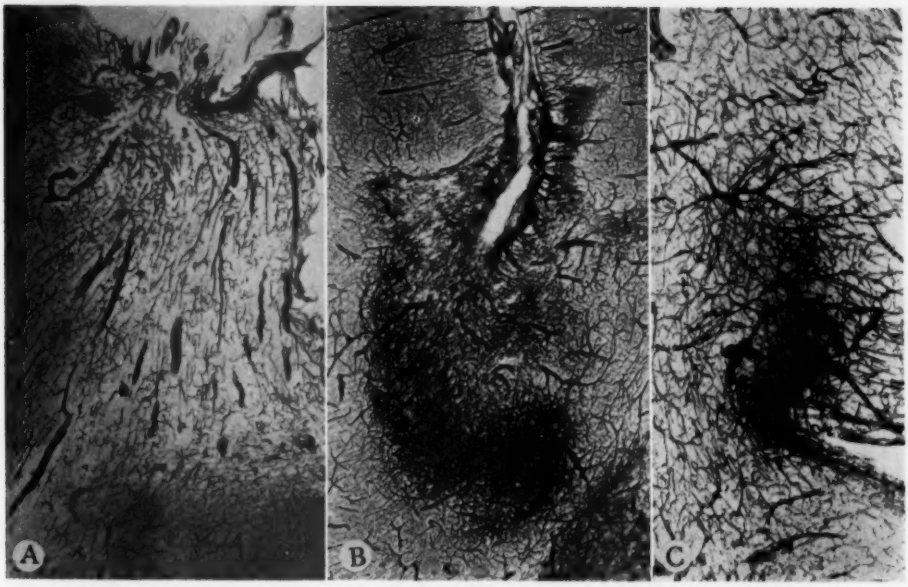


Fig. 3.—Structural types of scars. *A*, thin scar at margin of cortical defect resulting from gross loss of tissue; reduced about 20% from mag. × 8. *B*, highly vascularized scar in depth of sulcus; reduced about 20% from mag. × 12. *C*, small felt-work scar characterized by proliferated vascular net; reduced about 20% from mag. × 20. Mahoney preparations.

of limiting a vascular scar to a cortical defect with sharply outlined borders, it has been found that in the immediate vicinity of the defect a cluster of small scars can also be seen (Fig. 2). These "satellite" scars show a remarkable difference in their size and structural and architectural aspects. As far as their structure was concerned, the concept of three chief types as recognized in routine histological sections seem to be sustained. Minor focal ischemia in the cortical substance is manifested by a minimal vascular reaction with what appeared to consist of an increase in complexity of the vascular tree, a variability in the vascular pattern, and the number of capillary branches. These changes are often very limited in extent, resulting in a small, sharply circumscribed and tenuous scar (Fig. 3*A*). This change was usually associated with a regional increase in astrocytes and their transformation from the protoplasmic into the fibrous variety, although this reaction cannot be seen in these particular preparations. With early softening, the vascular reaction becomes more prominent, being characterized by a closely woven network of these elements accompanied by an early degree of proliferation of reticulin (Fig. 3*B*). But if the primary ischemia is profound and the affected nervous tissue has undergone total softening, the vascular reaction is again very limited and the ultimate scar (after phagocytosis and renewal of decadent material) consists of a very loose network of blood vessels and glial tissue (Fig. 3*C*). This special technique for the demonstration of blood vessels, therefore, does little more than to substantiate the present concept of the structure of these scars.

It was in the realm of morphology that this demonstration of the altered pattern in vascular scars is of greatest value. In the cases studied to date, the types of scars based on their architecture can be listed as follows: (1) superficial wedge-shaped scars, (2) vertical linear scars ("cascades"), (3) globular or cylindrical scars, (4) constricting or circumscribing scars, (5) extensive laminar or band-like scars, (6) small vascular

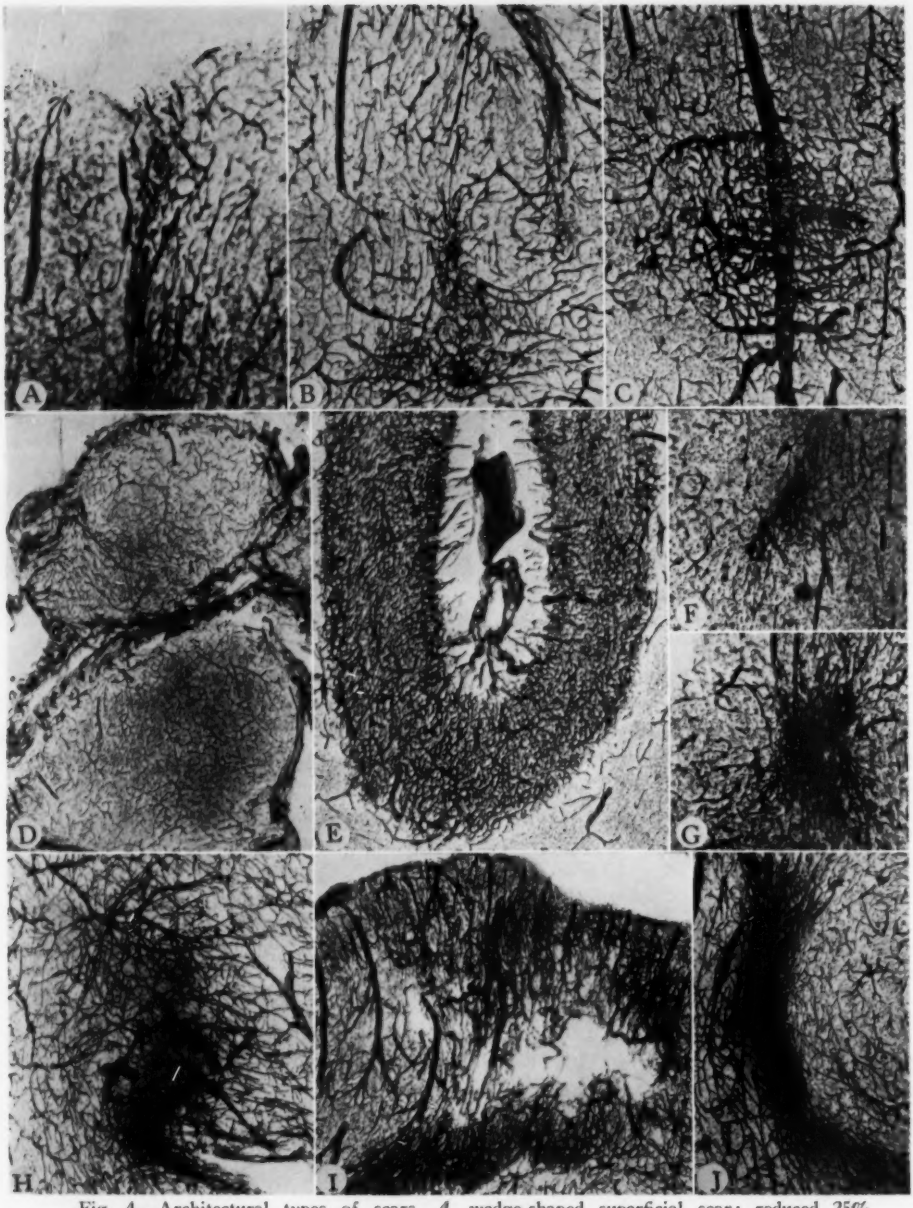


Fig. 4.—Architectural types of scars. *A*, wedge-shaped superficial scar; reduced 25% from mag. × 60. *B*, vertical ("pallisade") scar (above) and loose reticular scar (below); reduced 25% from mag. × 40. *C*, globular scar arranged about a large distended vein; reduced 25% from mag. × 20. *D*, constricting scar, isolating small focus of cortex; reduced 25% from mag. × 16. *E*, laminar scar occupying practically all cortical layers; reduced 25% from mag. × 20. *F*, fine nebulous scar composed of focal vascular rete; reduced 25% from mag. × 40. *G*, focal contractile scar; reduced 25% from mag. × 40. *H*, "brush-pile" scar; reduced 25% from mag. × 20. *I*, scar with central defect ("cortical cyst"); reduced 25% from mag. × 20. *J*, arcuate scar separating cortex and white matter; reduced 25% from mag. × 25. Mahoney preparations.

nebulosities, (7) brush-pile scars, (8) scars with central defects, (9) arcuate scars, and (10) loose reticular scars. A word about each of these morphological types and their possible significance is now in order.

Superficial Wedge-Shaped Scars.—These scars were invariably small and extended into the cortex in the shape of a wedge (in actuality a cone when considered in its three dimension), with its base at the pia mater (Fig. 4*A*). The larger scars of this type are poorly vascularized as a rule, being made up of a fibrous stroma continuous with, and adherent to, the leptomenix. Such scars are the presumed result of occlusion of one or more small penetrating arterioles.

Vertical Linear Scars.—Not infrequently one finds a long narrow scar made up of blood vessels arranged in parallel (Fig. 4*B*). At times such a scar can be traced upward to the pia mater, where it may be slightly curved, simulating in its total extent a tenuous waterfall or cascade. Some of these linear scars seem to extend deeply into the cortex but for the most part do not perforate it (section on constricting scars). These scars are the apparent result of an occlusion of a longer penetrating arteriole.

Globular or Cylindrical Scars.—These scars in a sense are the most interesting though less common lesions of the group. Thus far, in my experience, these scars appear to be composed of a complex of capillaries about a cortical venule which has a more than usual irregular contour and diameter. An unusual example of such a lesion is shown in Figure 4*C*.

Constricting or Circumscribing Scars.—This type of scar is longer than any of the above-mentioned varieties, is usually gently curving, and often extends from the surface of the cortex to the underlying white matter (Fig. 4*D*). It tends to constrict the cortex and isolate sections of the cortical gray matter. This process tends to produce visible constrictions or indentations on the surface of the convolution. Presumably, this type of scar represents obstruction of a long penetrating or a perforating arteriole.

Laminar or Band-Shaped Scars.—This type of vascular scar in its most prominent and characteristic form represents the end-picture of laminar sclerosis observed in persons who survive for any length of time a severe anoxemic insult (Courville).[10] As a rule this band of closely packed capillary blood vessels lies in the intermediate zone of the cerebral cortex, with a clear subpial and deep zone bordering it on either side (Fig. 4*E*). In some instances such a band may be limited in extent, joined on one aspect by a partially atrophic cortex and fading out on the other into the leptomeninges with complete disappearance of the gray matter. It must be recognized that what seems on section to be a band is actually a blanket extending in all directions for a variable distance.

Vascular Nebulosities.—These scars are very small and would very likely go undetected in routine preparations. They are of special interest in this connection, however, because their very limitation in size and depth permits one to have a third-dimensional concept of their structure (Fig. 4*F*). The loosely arranged vascular meshwork of which they are composed permits one to view its different levels by manipulation of the fine adjustment of the microscope. Fine fibrils of reticulin are seen emerging from the walls of these vessels, sometimes appearing like fine wires, at others like feathery tufts, at still others like twisted tendrils of a climbing plant.

"Brush-Pile" Scars.—These lesions consist of larger vascular nets, being centered around arterioles and venules rather than composed entirely of capillaries as is the case in the nebulosities (Fig. 4*G*). They too, however, are often rather loosely constructed, varying in this respect as well as their extent from one lesion to another. The orientation of this type of scar, as with the nebulosities, sometimes occurs without a notable tendency to either a vertical or horizontal arrangement.

Scars with Central Defects.—This lesion is usually manifest by a rather dense felt-

work of vessels with an open central cavitation (Fig. 4*H*), which involves practically the entire thickness of the cortex. It is therefore a veritable cortical cyst surrounded by a shell of scar tissue. The original lesion must therefore have been one with total destruction of a focal mass of cortical gray matter.

Arcuate Scars.—While all linear scars may be slightly curved at times, this particular lesion has a well-defined arcuate conformation. It usually lies in a horizontal position and parallels the lower margin of the cortex (Fig. 4*I*). It is difficult at times to determine whether this heavily vascularized scar actually lies within the deeper layers of the cortex or whether it is located between the cortex and white matter. It does not give one the impression of the result, however, of a laminar necrosis. It is not a common type of scar, judging from the material studied. It would seem to be a result of an occlusion of one or more of the arcuate subcortical arteries in this region.

Loose Reticular Scars.—These scars result from occlusion of larger arteries and involve both gray and white matter. They are composed of a very loose net of blood vessels and a thin feltwork of neuroglia (Fig. 4*J*). Such a tenuous scar must represent the end result of a complete ischemia with total destruction of everything but the regional vascular system itself not directly involved by the occlusion.

It may well be that further study of additional cases may disclose other morphological types of cortical scars. Moreover, transitions between types are apt to be found in the form of mixed scars, especially in the deeper layers of the cortex. In this region scars with a combination of vertical and horizontal orientation or those assuming an oblique direction are sometimes to be seen.

Vascular Components of Cortical Scars

With these various architectural types of cortical scars in mind, it is appropriate to turn to the alterations in the individual blood vessels to note their relation to the ultimate lesion. It is also worth while to note the possible changes in the nutrient arterioles, the capillaries, and the afferent system of veins.

Arteries.—In the various sections studied, significant structural changes in the small penetrating and perforating arteries of the cerebral cortex were not observed. This was as true of vessels in the vicinity of the scars as those remote from them. Wherever it was possible to trace one of these vessels into a scar, either a local or a widespread laminar one, the artery terminated abruptly, as though the seat of an occlusion (Fig. 5*A*). It was also noted that no perforating cortical artery passed through a laminar type of scar. By implication the subcortical white matter beneath an extensive laminar scar must have suffered from at least a relative ischemia.

Veins.—In contrast to the small arteries of the cortex, the corresponding veins undergo considerable changes in their sectional diameters and in their contours. Almost invariably the veins appear to be larger (Fig. 5*B*) and at times assume unusual proportions. Those emptying into the meningeal veins may become so large as to appear like sinusoids (Fig. 5*C*). The veins involved in the scar also presented an irregularity of contour beyond the normal. At times small bulbous excrescences were noted, as though the wall had become locally thinned and a miniature saccular or fusiform aneurysm had formed. Progressive disintegration of veins in some locations seemed to be taking place, but such vessels may have been inert from prior occlusion, because hemorrhages were not usually to be seen.

Capillaries.—Three different types of alterations in the capillaries were noted in the cortical scars examined in these sections. Most of these in the extensive laminar scars presented a fairly normal contour but were greatly condensed, with only a small amount of nervous tissue between their meshes. In the smaller scars the capillaries were more irregularly spread and some variation in the

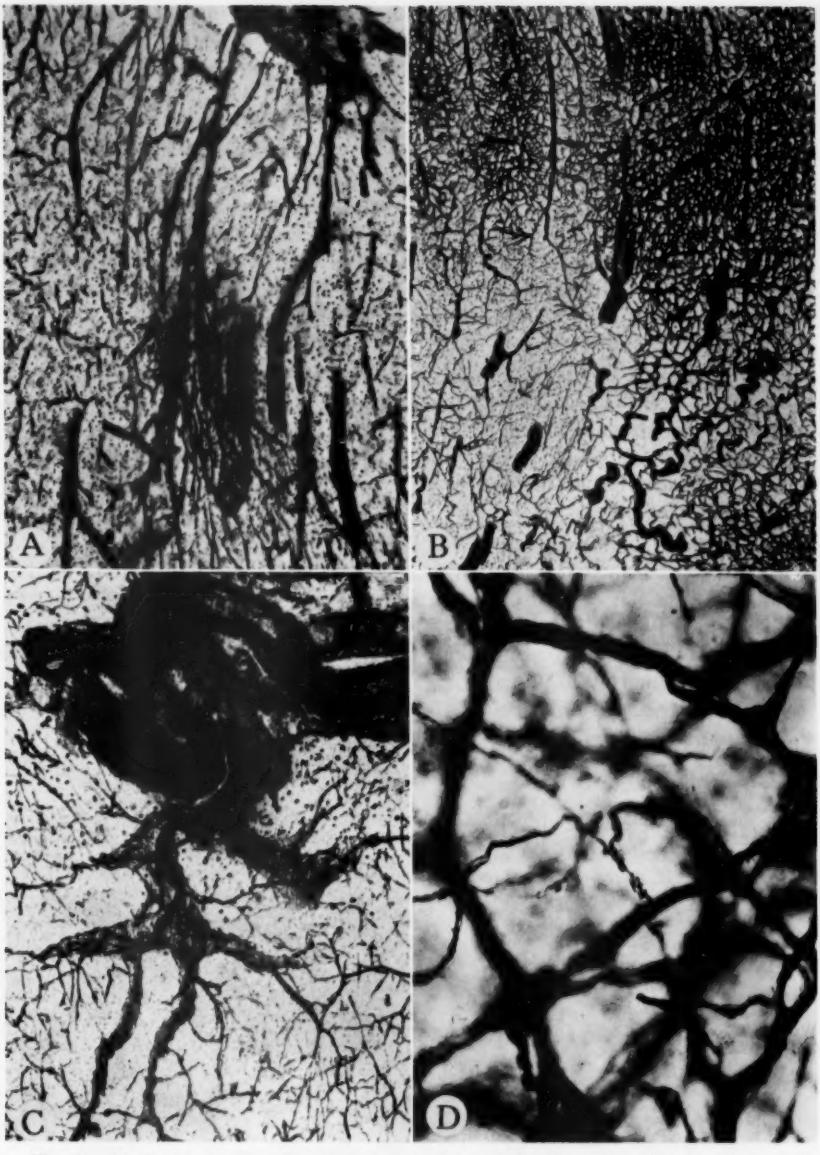


Fig. 5.—Alterations in vascular components of scars. *A*, abrupt termination of artery in scar; × 60. *B*, enlargement of veins within and at border of scar; × 30. *C*, enormously dilated superficial vein; × 30. *D*, alterations in caliber of capillaries in scar; × 20. Mahoney preparations.

diameter of their lumens was noted. Finally, it appeared as though a small segment of a capillary had collapsed and was reduced to a thin straight "string" of even thickness and smooth contour (Fig. 5*D*). In the smaller less uniformly vascularized scars,

the capillaries were somewhat more irregularly spaced and their original pattern considerably modified.

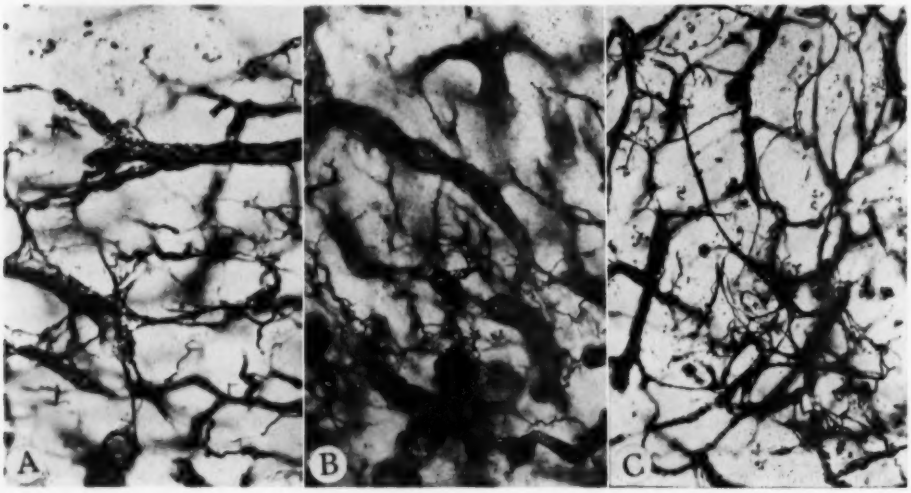
It now becomes apparent that a cortical scar of vascular origin is made up primarily of the blood vessels which were originally resident in the area at the time the arterial occlusion had taken place. The occluded artery, of course, terminated at this point, and the scar formed in the tissues supplied by it is distal to the point of obstruction. But most of the capillaries in this focus survive and form the basis for the ensuing scar. The blood cells coming from the adjoining vessels continue to pass through the system, being sluggishly evacuated by the veins. Why these veins should become so irregularly distended is not clear but such seems to be the case.

It may be asked, however, whether any other elements (other than the neuroglia) enter into the formation of these vascular scars. These studies indicate that there is a minor reaction on the part of reticulin. This reaction is most abundant at the borders of larger scars, particularly that region adjacent to the source of arterial supply. Here one can see small strands of reticulin reaching out into the intervals between the vessels either as a single fibril or a group of fibrils. In some instances an irregular net with variable size loops is formed. Such nets simulate broken-down spider webs. In some instances under higher magnification one can visualize very fine wavy, convoluted, or feathery extensions (Fig. 6). The amount of this reaction seems never to be very extensive and makes no major contribution to the structure of the vascular scar.

Summary and Conclusions

Cortical scars may be divided, from the view point of their etiology, into traumatic and vascular varieties. Contusions of the cortex usually result in simple defect scars; if the traumatic defect is complicated by bined astrovascular scar, with its tendency fragments of indriven bone and dura, a comto contracture and deformation of the surrounding nervous tissues, will form. Vascular scars, on the other hand, may be due either to arterial obstruction or to venous thrombosis. The arterial scars here considered are usually deficit scars with distortion only of the immediate cortex involved. These scars form a local capillary complex

Fig. 6.—Proliferation of reticulin. *A*, fine reticulin net at border of thin scar; reduced 10% from mag. × 160. *B*, proliferation of reticulin at border of laminar scar; reduced 10% from mag. × 180. *C*, focal net-like reticulin pattern as evidence of very delicate scar; reduced 10% from mag. × 160. Mahoney silver preparations.



if small and no great amount of nervous tissue is lost. However, with occlusion of a larger artery and breakdown of any sizeable mass of cortex, a thin glial scar with a reduction in the number of blood vessels results. These scars result from an occlusion of the penetrating cerebral arteries and assume a vertical orientation, thus constituting a local *vertical scar*.

Of particular interest is the occurrence, on an anoxemic basis, of a laminar scar which extends horizontally for variable distances in all directions, thereby forming a more or less extensive horizontal scar. This scar may be highly vascularized, being made up of a closely packed layer of capillaries, fed by the short cortical arteries, and drained by the venous afferents.

The changes in the blood vessels forming these scars are variable. The nutrient arteries show little or no alteration other than their sudden termination (occlusion) in the scar. In case of widespread laminar degeneration, the perforating arteries of the cortex are generally interrupted, resulting in a widespread relative ischemia of the underlying white matter. The capillary network may then become condensed into a veritable rete of vessels (laminar scar). In contrast a local reduction in their number constitutes a basis for a small contracting scar. The veins usually become dilated in an irregular fashion, in some instances forming large sinusoids draining the scar and its environs.

Aside from a proliferation and transformation of the resident neuroglia, in degrees dependent upon the degree and extent of tissue necrosis, the only other element found in formation of the residual scar is reticulin. This reaction is not of sufficient degree to alter materially the nature of the terminal scar.

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The Alkaline Phosphomonoesterase Activities of Brain Tumors

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Introduction

This study attempts to characterize the occurrence, distribution, and approximate intensity of the activity of three of the alkaline phosphomonoesterase groups of enzymes in brain tumors by histochemical means. These activities were correlated with the character and degree of malignancy of these tumors as indicated by parallel pathological preparations. While the material is of necessity limited in quantity, some basic patterns of enzymatic change are suggested.

The pattern of distribution of some of these enzyme activities in neural tissues [1-7] and in some brain tumors [1,8] has been previously described.

Material and Methods

The brain tumors, 62 in number, were all obtained surgically and were made available in the operating room promptly upon removal. The tissues were sectioned into slices 3 to 4 mm. in thickness; some portions were fixed in formalin for subsequent pathological examination and some fixed in cold acetone for the enzymatic studies. The acetone-fixed tissues were dehydrated, embedded in paraffin in a vacuum oven, sectioned, deparaffined, and rehydrated in the manner previously outlined.[4,9,10] A series of sections from the same block were immersed in the incubation mixtures described in Table 1 at 37 C for periods ranging from 5 minutes to 24 hours. They were then rinsed, and the enzymatically deposited calcium phosphate was visualized by the silver (von Kossa) technique.[10] Sections from the same acetone-fixed blocks were stained with hematoxylin and eosin so that the precise character of that portion of the tumor being studied histochemically could be recognized and the various loci of enzymatic activity correlated with their histologic characteristics.

TABLE 1.—*Incubation Mixtures* *

	Mixtures				
	A	B	C	D	E
Substrate, 33 mM.....	GP	GP	GP	MAA	MAA
Buffer, 200 mM.....	pd	pd	pd	pd	pd
Calcium, mM.....	13	13	13	13	13
Magnesium, mM.....	10	10	160	10	10
Cyanide, mM.....	0	10	0	0	10

* mM. indicates millimolar final concentration; GP, sodium β-glycerophosphate (Eastman Kodak Company); MAA, adenosine-5-phosphate (Schwarz Laboratories, N. Y.); pd, 2-amino-2-methyl-1,3-propanediol buffered at pH 9.2; calcium as nitrate, magnesium as chloride, cyanide as potassium salts.

Criteria for Delineation of Phosphomonoesterase Activities

Alkaline Phosphatase.—When preformed calcium or phosphate deposits were excluded, the brown or black deposits of silver noted in sections incubated in Mixture A (Table 1) were considered to indicate sites of alkaline phosphatase activity. Nuclear staining in this circumstance was evaluated as noted below.

Nuclear Phosphomonoesterases.—Nuclear staining noted in phosphatase preparations is the result of complex factors.[4,7,9-11] With the silver method of visualizing the calcium

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phosphate deposits, that artifactual staining related to the use of cobalt nitrate and ammonium sulfide is avoided.[10] There is, however, an artifactual nuclear staining due to the activity of enzyme adsorbed by nuclei after diffusion from other sites.[9] Enzyme activity in nuclei adjacent to sites of high cytoplasmic or extracellular activity and absent in identical cells further removed is considered the result of such an artifactual mechanism.

We hold that two forms of specific nuclear activity do occur. One of these is of low intensity, demonstrable only by the persistence of nuclear staining after long periods of incubation in the presence of 0.01 M cyanide or other inhibiting substances, in which circumstance the alkaline phosphatase activity in cytoplasmic and extracellular sites is inhibited.[7,11] Attempts to intensify this type of activity to provide valid information concerning its distribution and properties have not been successful. Because of the difficulty in distinguishing such activity from that due to enzyme adsorption, no effort will be made to characterize this activity in the tumors being described in this paper.

A separate type of nuclear phosphomonoesterase activity of limited distribution is manifest after short periods of incubation in the presence of high concentrations of magnesium ions, in which circumstance such activity is intensified in the nuclei in which it occurs, while the alkaline phosphatase activity in cytoplasmic and extracellular sites is partially inhibited.[11] Such activity was judged to be present when nuclear staining was noted after incubation for 30 minutes or less in the presence of 0.16 M magnesium (incubation Mixture C, Table 1), and nuclear staining was absent after incubation for a like period with 0.01 M magnesium (Mixture A, Table 1). The intensity of cytoplasmic staining is greater with 0.01 M magnesium (Mixture A, Table 1).

5-Nucleotidase.—This enzyme differs from alkaline phosphatase most specifically in that it hydrolyzes adenosine-5-phosphate and similar 5-nucleotides but does not hydrolyze sodium β-glycerophosphate or similar simple phosphate esters.[7,12] Alkaline phosphatase does split adenosine-5-phosphate with equal[7] or greater[13,14] rapidity than it splits glycerophosphate, and so the enzyme activity noted with adenosine-5-phosphate as substrate is the result of the activity of both enzymes, not of 5-nucleotidase alone. There are some who feel that activity demonstrated with adenosine-5-phosphate as substrate, after incubation for a period of time and under circumstances in which the substitution of glycerophosphate as substrate gives no staining, should be interpreted as 5-nucleotidase activity.[15] We consider this hazardous, since, as already indicated, alkaline phosphatase may split the adenosine-5-phosphate more rapidly than it splits glycerophosphate, and so its activity may be demonstrated with the former substrate in circumstances in which the degree of enzyme activity is too low to be demonstrated with the latter substrate. There are some who differentiate these activities by pH changes,[16] taking advantage of the fact that the optimum pH for 5-nucleotidase is lower than that for alkaline phosphatase,[16] although in other studies[13] the activity was equally intense up to pH 9.1. This advantage is negated by the fact that decreasing the pH increases the solubility of calcium phosphate, the deposition of which is utilized to indicate sites of enzyme activity. It is our experience that if attention is focused on sites known to contain 5-nucleotidase activity in the absence of alkaline phosphatase activity, such as the smooth muscle of arteries, such activity is manifest earlier and more intensely at pH 9.2 than at lower pH's.[7]

We prefer to utilize the fact that 0.01 M cyanide will inhibit alkaline phosphatase activity to a greater degree than 5-nucleotidase activity.[7] It will be recalled that with adenosine-5-phosphate as substrate, the staining is the result of the activity of both enzymes and the inhibition of one will

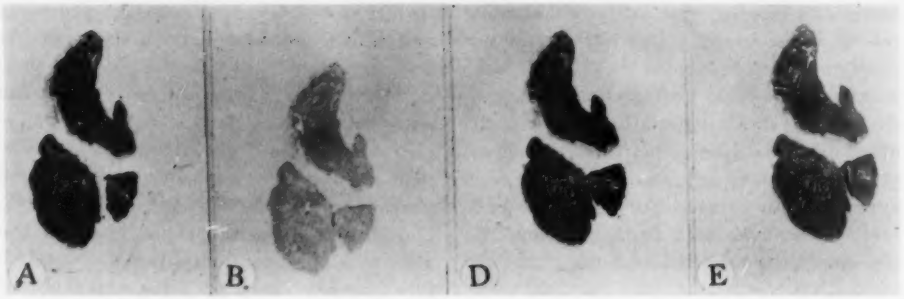

Fig. 1.—Meningioma. These replicate sections were incubated for 30 minutes in the incubation mixtures designated and are described in Table 1. *A*, the alkaline phosphatase activity demonstrated in the section incubated in Mixture A is markedly inhibited in *B*, the replicote section incubated in Mixture B, in which 0.01M cyanide was present. *D*, the section incubated in Mixture D with adenosine-5-phosphate as substrate reveals the activity of both alkaline phosphatase and 5-nucleotidase. *E*, the inhibition of the alkaline phosphatase activity by the cyanide in Mixture E discloses the activity of the 5-nucleotidase. Reduced 10% from mag. × 1.7.

permit an evaluation of the activity of the other. Unfortunately, cyanide will not completely inhibit alkaline phosphatase activity, nor is it completely without effect on 5-nucleotidase activity, and so the staining noted in the presence of cyanide need not necessarily be the result of the latter alone. We have arbitrarily accepted as a criterion the principle that we can be certain of the presence of 5-nucleotidase activity, if the intensity of staining in an area is greater with adenosine-5-phosphate in the *presence* of cyanide (incubation Mixture E, Table 1) than with glycerophosphate in the *absence* of cyanide (incubation Mixture A, Table 1). It is recognized that with these rigid criteria, 5-nucleotidase activity is not likely to be disclosed in the presence of a very great alkaline phosphatase activity. In the present study, this situation arose only with respect to the meningiomas, some of which were very highly active with respect to alkaline phosphatase activity. Since the degree of inhibition by cyanide in this circumstance was far greater when sodium-β-glycerophosphate served as substrate (Mixtures A and B, Table 1) than when adenosine-5-phosphate was utilized (Mixtures E and F, Table 1), it is considered likely that 5-nucleotidase was also present in these tumors (Fig. 1).

Observations

Normal Tissues.—In six instances, the surgical procedure required the removal of normal neural tissues. The alkaline phosphatase activity was essentially like that previously described, being interstitial and appreciably greater in the gray than in the white matter. In one instance, an accentuation of the activity in the internal granular layer of the cerebrum was apparent. In the cerebellum, the alkaline phosphatase activity in the molecular layer of the cortex was greater than in other portions of the cortex or in the white matter. The 5-nucleotidase activity of the cerebellar white matter was greater than that of any portion of the cerebellar cortex, while in the cerebrum this activity was still greater in the cortex than in the white matter, although the difference was considerably less than was true with respect to alkaline phosphatase.

Blood Vessels.—Most blood vessels in normal tissues and normal vessels in neoplastic tissues revealed a moderately active alkaline phosphatase activity on the endothelial surface. The activity in the hyperplastic vessels in some of the malignant tumors, particularly the glioblastoma, revealed a less intense activity but one which was more widely diffused among the cells forming the hyperplastic wall. No 5-nucleo-

tidase activity was demonstrated in these hyperplastic vessels or in normal capillaries, but the smooth muscle of arteries clearly revealed the presence of such activity. There was no magnesium-activated nuclear activity. No further reference will be made to activity in blood vessels in the descriptions which follow, these descriptions being limited to the parenchymal and neoplastic tissue elements and, where appropriate, to the stroma.

Astrocytoma-Glioblastoma Group.—There were 21 tumors of the astrocytoma-glioblastoma group [17] of which four were of slight degrees of anaplasia and might be considered as astrocytomas. The others were of moderate to marked degrees of anaplasia warranting the designation of glioblastoma. The alkaline phosphatase activity in all was considered slight or moderate in intensity. There was no consistent relationship between the intensity of alkaline phosphatase activity and the degree of anaplasia. The 5-nucleotidase activity was absent or slight in the tumors of low anaplasia, but in 10 of the highly malignant tumors such activity was clearly demonstrable, and it was present presumptively in three others. One of the malignant tumors was a composite glioblastoma and sarcoma,[18] but none of the sarcomatous tissues was represented in the materials studied histochemically. It may be noted that enzyme activity persisted in the necrotic debris of the glioblastoma in circumstances in which the necrosis appeared fresh but was lost in the necrotic zones of older duration. There was no magnesium activated nuclear activity.

Other Gliomas.—There were four ependymomatous tumors available for study, one of which was markedly anaplastic. The three less malignant tumors revealed a slight to moderate alkaline phosphatase activity in the tumor cells, and in one this was somewhat greater in the vicinity of blood vessels than in the tissues distant from the vessels. There was no 5-nucleotidase activity. The fourth tumor consisted largely of a highly anaplastic undifferentiated tissue with areas which showed the characteristic perivascular formations of the more differentiated ependymoma. This tumor revealed only a slight alkaline phosphatase activity but a moderate 5-nucleotidase activity.

There was one oligodendroglioma which revealed a moderate alkaline phosphatase activity and no 5-nucleotidase activity. This tumor also contained astrocytomatous areas and the two tissues were essentially similar enzymatically.

There were two medulloblastomas, in one of which the alkaline phosphatase activity

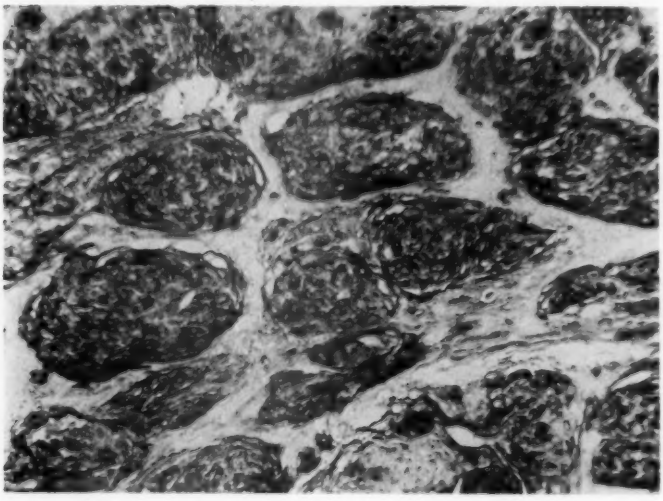

Fig. 2.—Meningioma. The section was incubated for 30 minutes in incubation Mixture A. Alkaline phosphatase activity is demonstrated in whorls and in meningothelial tissues. Reduced about 35% from mag. × 100.

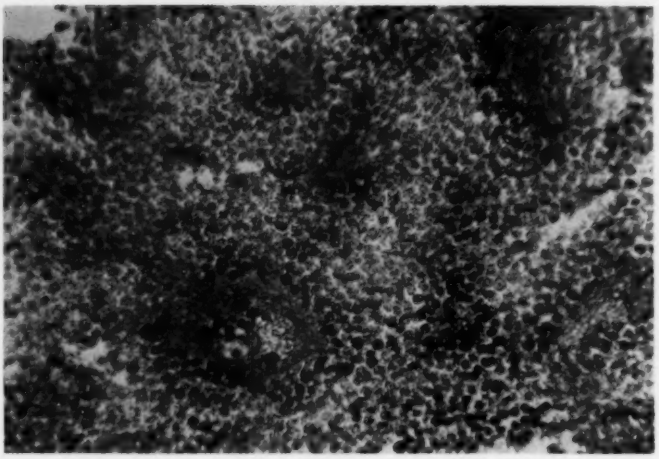

Fig. 3.—Neuroblastoma. This section was incubated for 24 hours in incubation Mixture A. The alkaline phosphatase activity is greater in the rosette than in the intervening tissues. The nuclear staining is presumably the result of enzyme adsorption. Reduced 40% from mag. × 250.

was moderate and in the other, slight in degree. There was no 5-nucleotidase activity.

There was one neuroblastoma of the perispinal tissues which very closely resembled the medulloblastoma histologically and enzymatically. It showed a moderate alkaline phosphatase activity and no 5-nucleotidase activity. In the neuroblastoma the alkaline phosphatase activity was appreciably greater within the rosette than in the surrounding tissues (Fig. 3).

There was no mangesium activated nuclear activity in any of these tumors.

Meningioma.—Thirteen meningiomas were studied, one of which was considered malignant; one, angioblastic,[19] and the others, composed of admixtures of meningothelial and fibroblastic components often with the characteristic whorl. The malignant meningioma revealed little, if any, alkaline phosphatase activity, but there was significant 5-nucleotidase activity, particularly in the fibrotic stroma. The angioblastic meningioma, a benign neoplasm, revealed a pattern like that of the malignant meningioma, and the same was true of one other, an essentially meningothelial meningioma.

A moderate alkaline phosphatase activity was demonstrated interstitially in six meningiomas, in four of which an appreciable 5-nucleotidase activity was also demonstrated. In one of the others, no 5-nucleotidase activity was demonstrated, while the sixth was not tested for this enzyme. These tumors were composed of meningothelial and fibroblastic elements in varying proportions, but these tissue elements did not differ consistently in their enzymatic properties. Large, relatively poorly defined whorls were present in four of these tumors and tight concentric whorls in the fifth, but these were not enzymatically distinguished.

The other four meningiomas demonstrated an intense alkaline phosphatase activity, more marked than in any other brain tumor. In three of these there were numerous small tight concentric whorls, and it was clear that these were appreciably more active than the intervening tissues (Fig. 2). The other highly active tumor was essentially meningothelial in character. In these four tumors the presence of 5-nucleotidase is difficult to evaluate because of the intensity of the alkaline phosphatase activity, as discussed above under "Criteria for Delineation.". As indicated there, the fact that the degree of inhibition by cyanide was far greater with sodium beta glycerophosphate as substrate than with adenosine-5-phosphate as substrate, suggests that 5-nucleotidase was present in these as well (Fig. 1).

Nerve Sheath Tumors.—There were seven tumors classified as nerve sheath tumors, all

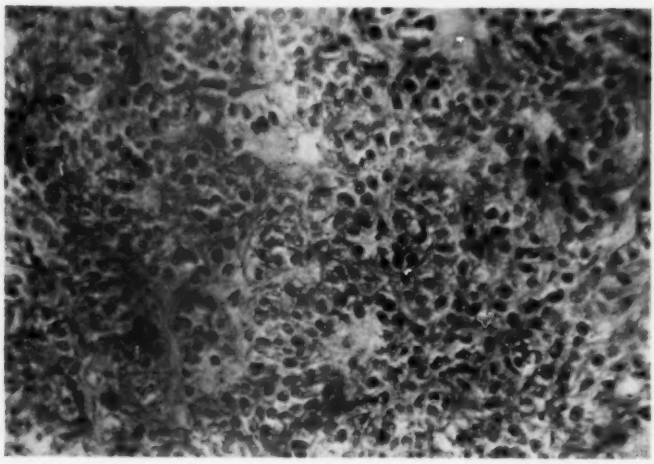

Fig. 4.—Pituitary carcinoma. The section was incubated for 10 minutes in Mixture C, containing 0.16 magnesium ions. Such nuclear activity was not evident in a replicate section incubated in Medium A, containing 0.01 magnesium. Reduced 40% from mag. × 250.

benign. We prefer not to attempt to subdivide this group of tumors into neurofibromas, neurolemmomas, etc., as preferred by others. In four instances a slight interstitial alkaline phosphatase activity was demonstrated, and this was moderate in the other three. In the latter it was apparent that the denser, more highly cellular areas showed a greater activity than the loose meshed zones, and areas of palisade formation were particularly active (Fig. 5); 5-nucleotidase was absent or minimal in four and slight in three. The latter were not identical with those in which the higher degree of alkaline phosphatase activity was noted.

Choroid Papilloma.—There was one such tumor in which alkaline phosphatase activity was demonstrable in the blood vessels and to less degree in the interstitial connective tissues. Studies for the other enzyme activities were not performed.

Pituitary Tumors.—Three chromophobe pituitary tumors were available for study, one of which showed no anaplasia; the second showed a slight to moderate degree of anaplasia, while in the third the degree of anaplasia was so marked as to warrant a diagnosis of pituitary carcinoma. There was no alkaline phosphatase or 5-nucleotidase activity in those tumors with no or

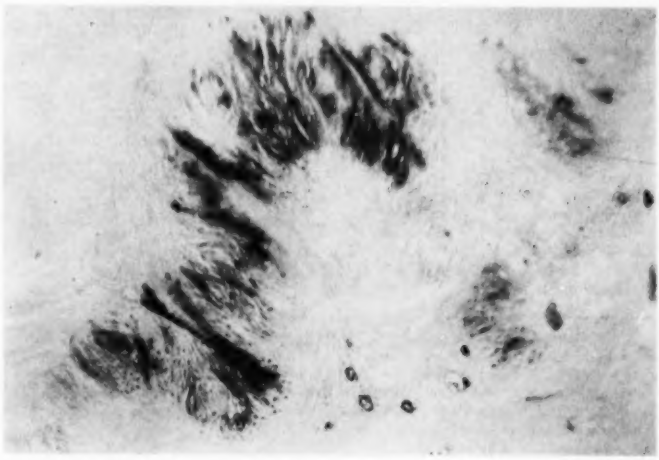

Fig. 5.—Nerve sheath tumor. This section was incubated in Mixture A for three hours. Alkaline phosphatase activity is greater in the dense tissues forming a palisade than in other portions of the tumor. Reduced 40% from mag. × 100.

moderate anaplastic changes. The pituitary carcinoma revealed a moderate diffuse alkaline phosphatase activity and an appreciably greater 5-nucleotidase activity in the tumor cells. In this instance also, magnesium-activated nuclear activity was clearly demonstrated in the tumor cells (Fig. 4).

Metastatic Tumors.—There were nine tumors metastatic to the brain, including one melanoma and eight carcinomas. The melanoma revealed only a slight alkaline phosphatase activity in the connective tissue stroma and no 5-nucleotidase activity. In five of the other cases, a slight to moderate alkaline phosphatase activity was limited to the connective tissue stroma, the carcinomatous cells themselves showing no activity. In one instance, an adenocarcinoma revealed in addition activity in the lumenal border of some of the acini, while in two cases of epidermoid carcinoma the cytoplasm and nuclei of the carcinomatous cells revealed phosphatase activity; 5-nucleotidase activity was demonstrable in the connective tissue stroma in all but the melanoma, though this was relatively slight in three. In one instance, a highly anaplastic carcinoma, magnesium-accentuated nuclear activity was evident in the carcinoma cells. In this instance, no alkaline phosphatase or 5-nucleotidase activity was present in these cells.

TABLE 2.—*Summary of Observations*

	Cases, No.	Alkaline Phosphatase *	5-Nucleotidase *	Nuclear Phosphomonoesterase *
Astrocytoma	4	+-++	0-+	0
Malignant astrocytoma	17	+-++	+-++	0
Ependymoma	3	+-++	0	0
Malignant ependymoma	1	+	++	0
Oligodendroglioma	1	++	0	0
Medulloblastoma	2	+-++	0	0
Neuroblastoma	1	+	0	0
Meningioma	10	+-+++	+	0
Meningioma †	3	0	+	0
Nerve sheath tumor	7	+-++	0-+	0
Pituitary adenoma	2	0	0	0
Pituitary carcinoma	1	++	++	+
Choroid papilloma	1	+		
Metastatic carcinoma	9	0-+	0	+‡
Stroma of carcinoma		+-++	0-++	0

* 0 indicates essentially no activity; +, slight activity; ++, moderate activity; +++, marked activity.

† This group included 1 malignant meningioma, 1 angioblastic meningioma, and 1 meningothelial and fibroblastic meningioma not distinguishable histologically from those in which alkaline phosphatase was active.

‡ Demonstrable in only 1 of 9 cases.

Comment

These observations are summarized in Table 2. The number of cases is small, and while an enzymatic pattern is suggested with respect to some groups of tumors, this can hardly be considered as clearly demonstrated. In the astrocytoma group, increasing degrees of malignancy [17] appear to be associated with an increase in 5-nucleotidase activity, and this may be true as well of the ependymoma group. The neuroblastoma and the medulloblastoma, both highly malignant gliomas, did not reveal a similarly high 5-nucleotidase activity, more closely resembling the benign astrocytoma and oligodendroglioma in enzymatic pattern. The pituitary tumors, though small in number, showed a similar increase in 5-nucleotidase activity with increased malignancy, and this was associated with increased alkaline phosphatase activity as well, a feature not evident in the gliomas. The meningiomas in general revealed high alkaline phosphatase activity, although this was not true of a malignant meningioma, an angioblastic meningioma, and one other meningioma, neither malignant nor angioblastic. It is of interest that the angioblastic meningioma often contains bizarre cells like those seen in malignant tumors [19] and, while recognized to be benign, was once thought to be more aggressive. The activity of the metastatic carcinomas was located primarily in the stroma, but the carcinoma cells were active in a few instances.

In most instances the enzyme activity is diffusely distributed in the tumor tissues without being specifically localized to any structure. In a sense this parallels the histologic appearance of many brain tumors, which are not often characterized by discrete recognizable cell formations. However, alkaline phosphatase activity did appear to be specifically increased in the rosette of the neuroblastoma, the tight whorl

of the meningioma, and the palisade of the nerve sheath tumor and possibly in the perivascular formation of the ependymoma.

The magnesium-activated nuclear activity could be demonstrated in only two instances, a metastatic carcinoma and the pituitary carcinoma. It may be noted that this enzyme appears to be of very limited distribution, being most clearly demonstrable in the endometrial and the ovarian stroma.[11]

Summary

Sixty-two brain tumors were studied pathologically and histochemically for the activity of alkaline phosphatase, 5-nucleotidase, and the magnesium-activated nuclear phosphomonoesterase. The criteria for the histochemical delineation of these enzyme activities, distinguishing each in the presence of the others, were established, and the description of the enzymatic pattern of the tumors was based on these criteria. There was a tendency for the 5-nucleotidase activity of the astrocytoma, ependymoma, and pituitary groups of tumors to increase with increasing degrees of malignancy, while in the meningioma group a decrease in alkaline phosphatase activity occurred in this circumstance. A few specific histologic structures revealed appreciably greater alkaline phosphatase activity than the adjacent portions of the tumors in which they were contained, notably, the rosette of the neuroblastoma, the tight concentric whorl of the meningioma, the palisade of the nerve sheath tumor, and possibly the perivascular formation of the ependymoma. The magnesium activated nuclear phosphomonoesterase activity was demonstrated in only two tumors, a metastatic carcinoma and a pituitary carcinoma.

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Cellular and Intercellular Changes in the Arthus Phenomenon

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The experiments to be reported deal with the cellular and intercellular changes (1) in the Arthus phenomenon, (2) in the Arthus phenomenon elicited in a passively sensitized animal, and (3) following a single intracutaneous injection into a nonsensitized rabbit.

In contrast to the opinion of Rössle,[26,27] the observations to be reported show that the tissue reaction in the active Arthus phenomenon is not only quantitatively but also qualitatively different from a normergic or ordinary inflammation and the reaction pattern is highly characteristic for an active Arthus lesion. Furthermore, plasma cells which appear early in this reaction, but are absent in this phase of the passive Arthus, represent the local production of antibody, while macrophages which are a prominent feature of the active Arthus, passive Arthus, and the response to a single antigen injection remove antigen from the interstitial tissue. Finally, the abundant serous and serofibrinous exudate which is prominent in the early phase of both the active and passive Arthus lesions appear to be an acute inflammatory response to antigen-antibody union.

A. The Arthus Reaction

The primary object of all the experiments of this paper was to determine the characteristic or pathognomonic features of tissue reactions in local anaphylactic hypersensitivity and the functional significance and pathogenesis of the various components of the lesion. The first part of the study was therefore concerned with defining the sequence of morphologic changes occurring in the Arthus reaction.

Material and Methods

Twenty-three albino rabbits weighing between 2,000 and 2,500 gm. were used in these experiments. They were fed Purina Rabbit Chow and water ad libidum. All animals were killed with an intravenous injection of pentobarbital (Nembutal) sodium, and the tissues where the antigen had been injected were excised and fixed in 10% buffered formalin[19]; some also in Bencosme's[2] Zenker-formol variant and in formol-sublimate-acetic. Some of the joint material was fixed in 1% trichloracetic acid in 80% ethanol. The joints were decalcified in 5% formic acid. Following dehydration and clearing, the tissues were embedded in paraffin and yellow wax (beeswax) and cut at 3μ-5μ. All sections were stained with hemalum-phloxine-saffron[5] and methyl green-pyronin (MGP). Selected sections were stained by the following staining methods: Masson's trichrome; Pentachrome I and II,[21] Mallory's phosphotungstic acid hematoxylin (PTAH); Lillie's allochrome,[19] and May-Grünwald-Giemsa. A series of histochemical tests were also carried out: Periodic acid Schiff (PAS) (with or without orange G in phosphotungstic acid counterstain); toluidine blue; Alcian blue (AB)-nuclear fast red; the Millon; the Voisenet-Furth; the Barnett-Seligman; methyl green-pyronin (with and without ribonuclease incubation), and the Feulgen reactions. In those experiments dealing with the passive Arthus and the response to an initial injection of antigen, the animals were killed and tissues fixed and stained in the same way.

Experiment I.—Ten animals received on the first day of the experiment 10 ml. of Seitz filtered horse serum per kilogram of body weight intravenously. Sixteen days later they were given 0.1 ml. of antigen intracutaneously. The animals were

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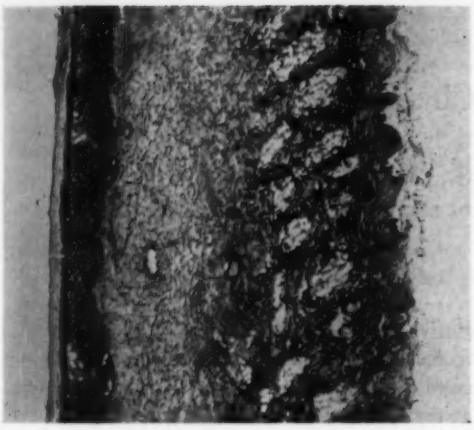

Fig. 1.—Arthus reaction of skin, 24 hours; dermis with underlying layer of striated muscle; dermis is swollen, having three to four times the thickness of normal skin; some vessels are partly thrombosed. Trichrome; × 11.4.

killed in groups of two at the following intervals: 24 hours and 2, 5, 8, and 14 days after the local injection of antigen.

Experiment II.—Seven animals were given 2 ml. of sterile horse serum intravenously on Day 1; 25 days later they received 10 ml. per kilogram of body weight intravenously, and eight days after this massive dose they were given injections intra-articularly with 1 ml. of horse serum. They were killed in the following order after the last injection: 24 hours, 3 days (one animal each), 5 days and 9 days (two animals each), and 14 days (one animal). Strips of the joint capsule including the synovialis were placed in the fixative. In addition, the whole joint was fixed, decalcified, and cut as a whole from each of one of the animals killed on Days 5 and 9.

Experiment III.—Six animals were pretreated with three intravenous injections of horse serum: 5 ml. was given to each animal on Days 1, 4, and 7. On Day 18 they were given injections of 2 ml. of horse serum into the left pleural cavity. They were killed at serial time intervals in the following order: 24 hours and 3 days (one animal each), 5 days (two animals), 9 and 14 days (one animal each).

Morphological Observations

Skin.—Twenty-Four Hours: An acute exudative inflammation with large amounts of serous fluid widely separated the connective tissue elements of the dermis (Figs. 1 and 2). Collagen fibers were fragmented. Although a large number of mononuclear cells were present, polymorphonuclear cells and pseudoeosinophils predominated. At this stage large plasmoblasts could be demonstrated around vessels of the loose areolar connective tissue along the layer of striated muscle of the skin.

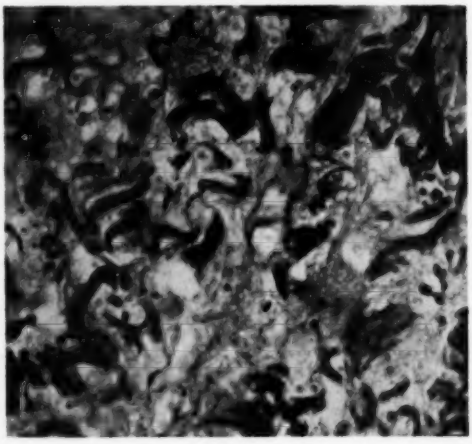

Fig. 2.—High power of Figure 1. Collagen fibers are widely separated by serofibrinous exudate. (Meshwork of fibrin is seen in gray.) Trichrome; × 184.

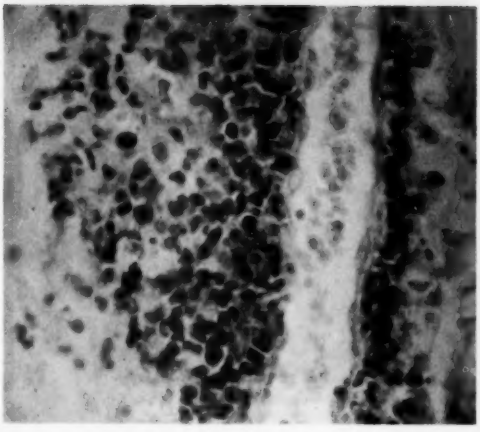

Fig. 3.—Arthus of skin, three days; accumulation of immature and mature plasma cells along vein at the base of dermis. (The characteristics of these cells are seen in Figures 4, 6, and 16.)

Thirty-Six Hours: The exudate had regressed and serous fluid was no longer demonstrable. Polymorphonuclear cells and pseudoeosinophils were still present. Mononuclear cells became prominent at this time. They consisted of monocytes, histiocytes, occasional lymphocytes, and proliferating fibroblasts. Immature and mature plasma cells were numerous around vessels, i. e., at sites where plasmoblasts were seen in the 24-hour sections (Fig. 3).

Five Days: The lesions differed from those of three days by showing a less intense polymorphonuclear and proportionately more intense mononuclear reaction. Most of the plasma cells seen at this stage were mature, although a large number of immature forms was still present.

Nine Days: In the lesion of one of the animals there was an area of central necrosis composed of disintegrating cells, tissue, and nuclear debris. In this debris, but more so in the granulation tissue surrounding this central mass, there were numerous macrophages with cytoplasmic vacuoles. The granulation tissue consisted of proliferating fibroblasts, newly formed capillaries, occasional pseudoeosinophils, and, at the periphery, a wall of mature plasma cells.

Fourteen Days: One of the animals showed a central mass of granulation tissue. In this granulation tissue, but more at its periphery, numerous mature plasma cells were found. There was no granulation tissue in the second animal, but there was a mild cellular reaction at the border of

Fig. 4.—Arthus of skin, 14 days; mature plasma cells between collagen fibers. Pentachrome II; × 775.

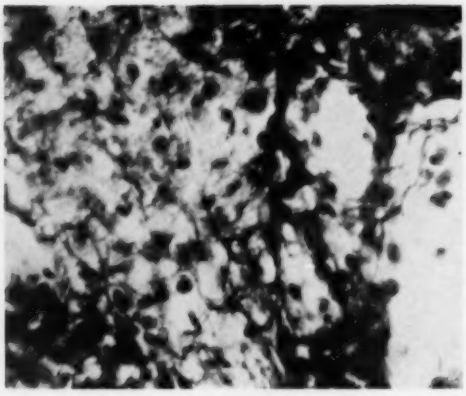

Fig. 5.—Arthus of joint, 24 hours; fibrinous exudate and fibrinoid in synovialis. The fine black fibrils and conglomerated masses represent PTAH-positive (dark blue) fibrin and fibrinoid. Phosphotungstic acid hematoxylin; × 307.

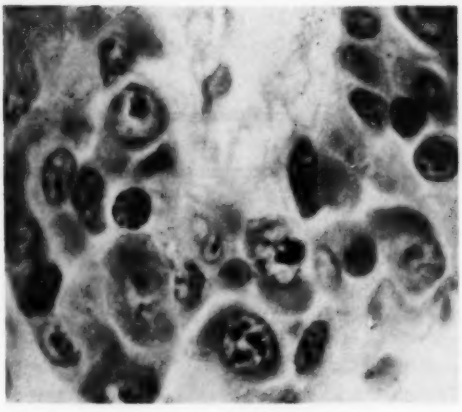

Fig. 6.—Arthus of joint, 24 hours; immature plasma cells around venule in synovialis. May-Grünwald-Giemsa stain; × 1,464.

dermis and subcutaneous tissue. Many of these cells were plasma cells (Fig. 4).

Joint.—Twenty-Four Hours: Extensive serous exudation involved all the connective tissue elements of the capsule, but particularly that of the synovial tissues. Acid mucopolysaccharides (AMP) of the synovialis were diminished in areas of extensive

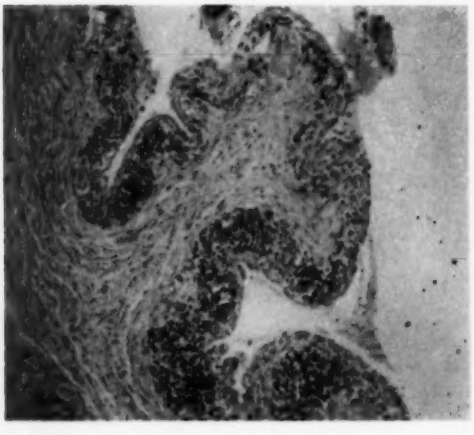

Fig. 7.—Arthus of joint, 72 hours; proliferation of numerous cells in synovial villi below the lining mesothelial cells. Under high power the majority of these cells had all the characteristics of mature and immature plasma cells. May-Grünwald-Giemsa stain; × 77.

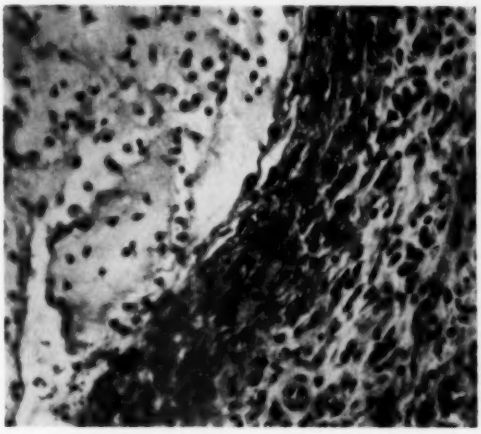

Fig. 8.—Arthus of joint, five days; serofibrinous exudate and synovial fluid in joint cavity. Wavy bands and clumps of fibrinoid in thickened synovialis. Most cells are macrophages. Alcian blue—periodic acid Schiff—orange G; × 184.

serous exudation. Collagen fibers were widely separated but showed no "necrosis." Exuded fibrin was seen in one or two cases in the synovialis as delicate blue fibrils in the PTAH stain (Fig. 5), as well as in the wall and perivascular tissue of several arteries. Some vessels showed partial thrombosis. The inflammatory cells consisted mainly of polymorphonuclear leukocytes and pseudoeosinophils, although mononuclear cells were very numerous. Besides proliferating histiocytes, monocytes, and fibroblasts, there were other large mononuclear cells present, distributed diffusely, in small numbers or in large groups around vessels (Fig. 6). These cells could be identified as plasmoblasts, which resembled those in the skin.

Seventy-Two Hours: The acute inflammatory reaction had almost subsided with only a moderate number of polymorphonuclear cells and pseudoeosinophils present. There was, however, a rather intense mononuclear proliferation. The synovial lining cells were prominent. Proplasmocytes and plasmoblasts were seen in large numbers in the subsynovial loose connective tissue of villi (Fig. 7) and around vesels.

Five Days: Abundant protein-rich exudate was present on the synovial surface and in the joint cavity, and intensely acidophilic wavy bands and clumps of fibrinoid were also present in the synovialis (Fig. 8). The fibrin was intermingled with a homogeneous or faintly granular pink-staining material. Some faintly hematoxylinophilic material

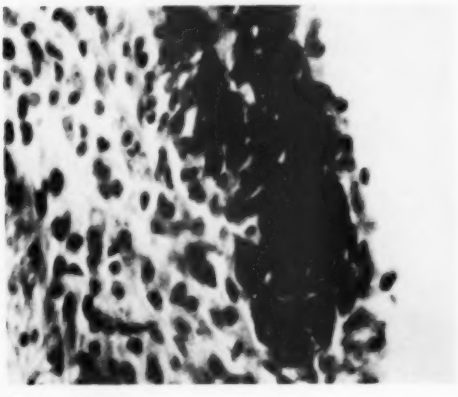

Fig. 9.—Arthus of joint, nine days; clumps of fibrinoid in synovialis staining dark purplish-blue with phosphotungstic-acid hematoxylin (black in photograph); × 307.

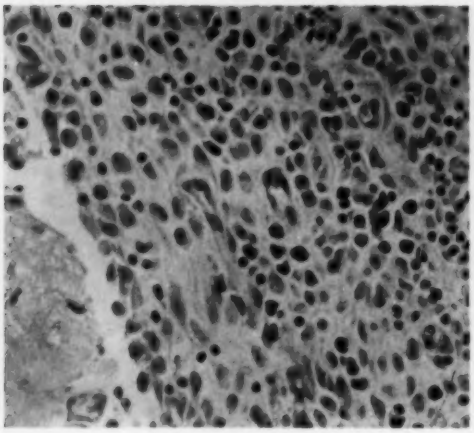

Fig. 10.—Arthus of joint, nine days; granulomatous reaction of synovialis. Cells in the lesion are macrophages (pale round cells), fibroblasts (pale elongated cells), and plasma cells (dark cells). Inspissated serous exudate is seen in the joint cavity, in the left lower corner of photograph. Hemalum-phloxine-saffron; × 307.

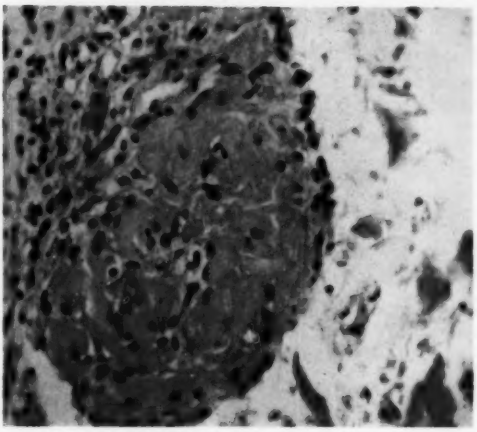

Fig. 11.—Arthus of joint, nine days; inspissated serous exudate adherent to synovialis, covered by mesothelial cells and invaded by organizing cells. Hemalum-phloxine-saffron; × 246.

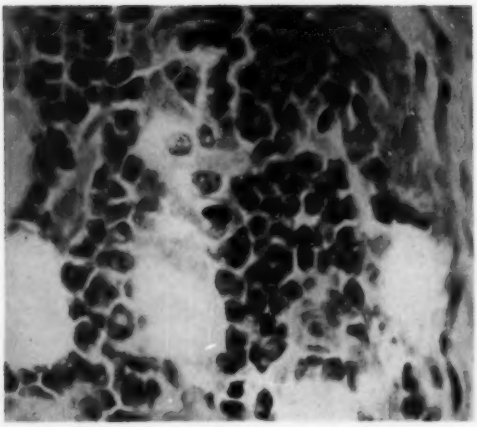

Fig. 12.—Arthus of joint, nine days; numerous mature plasma cells in loose adipose tissue of synovialis; note eccentric nucleus and juxtanuclear halo. Methyl green-pyronin; × 410.

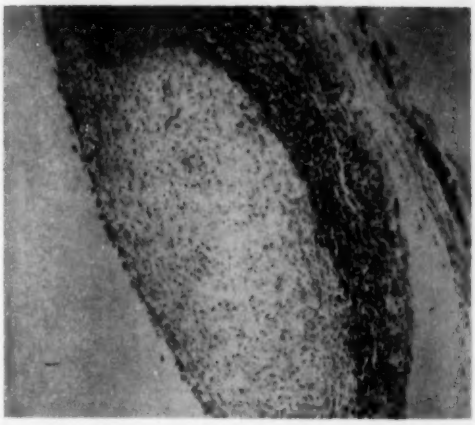

Fig. 13.—Arthus of joint, 14 days; organized exudate (pale oval area) surrounded by numerous plasma cells. May-Grünwald-Giemsa stain; × 77.

was also present in the joint cavity. In PTAH only the fibrin and fibrinoid was dark blue. The majority of the cells in the synovialis were marcrophages, fibroblasts, and plasma cells. The latter were found mostly in small groups or as perivascular cuffs. By now mature forms of plasma cells were more numerous than their precursors.

Nine Days: There was an abundant and extensive cellular reaction and deposition of fibrinoid (Fig. 9). Large areas of loose areolar tissue of the synovialis had a highly cellular granulomatous appearance (Fig. 10). Whereas some of the inspissated serous exudate still lay in the joint cavity (Fig. 10), some was already incorporated into granulation tissue. Synovial cells covered the protein coagulum, and fibroblasts and histiocytes had invaded it (Fig. 11). The newly formed fibers present in the organizing granulomatous tissue were all Alcian blue-positive and faintly metachromatic with toluidine blue. Some of the plasma cells present in this tissue were large immature forms, but the majority were mature plasma cells with eccentrically situated "cartwheel" nuclei, with the chromatin at the periphery of the nuclear membrane. Many of them contained juxtanuclear halos (Fig. 12).

Fourteen Days: Most of the exudate was organized. The clumps of inspissated protein seen in the nine-day sections (Fig. 11) were entirely replaced by cells, i. e., histiocytes and fibroblasts (Fig. 13). The cellularity of the tissue had decreased. The cells consisted of histiocytes, lymphocytes, fibro-

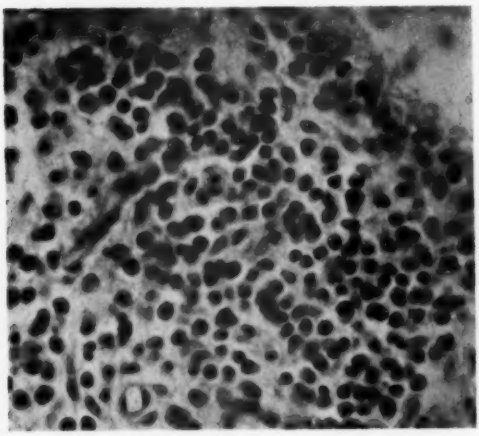

Fig. 14.—Arthus of joint, 14 days; proliferating mature plasma cells in synovialis. Mesothelium in right upper corner. Hemalum-phloxine-saffron; × 307.

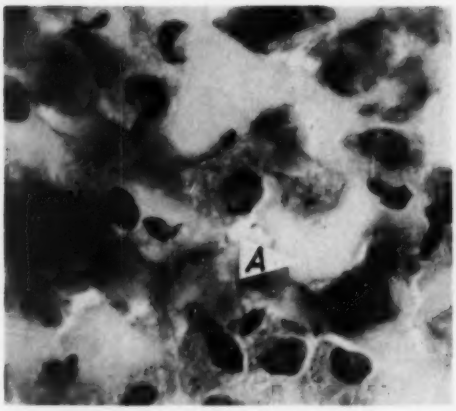

Fig. 15.—Lesion of alum-precipitated horse serum; five days. Macrophages are seen between fragments of collagen fibers and the homogeneous light gray antigen (*A*). Unfixed frozen section; trichrome; ×775.

blasts, and plasma cells. The plasma cells were mainly mature forms (Fig. 14).

Pleura.—The lesion in the pleura was basically the same as in the joint and skin.

At 24 hours the inflammatory exudate covered the pleura, and the lung beneath the affected pleura showed hyperemia, extravasation of red cells, and a mononuclear proliferation. The tissues under the parietal pleura were also involved in the inflammatory process. By the third day the acute inflammatory reaction had regressed but some exudate made up of protein-rich fluid and cellular debris still lay on the pleural surface. In the lung beneath the pleural lesion, desquamated cells, erythrocytes, and serofibrinous fluid lay in alveoli. The lesion resembled rheumatic pneumonitis, particularly in areas where fibroblasts had invaded the exudate. Immature and mature plasma cells were present in the thickened pleura and as perivascular cuffs in the lung. On the fifth day, the exudate was almost completely organized, except for a fairly circumscribed area where varying sized clumps and masses of intensely acidophilic fibrinoid lay surrounded by a wall of granulation tissue. On the ninth experimental day most of the exudate had become firmly attached and incorporated into the pleural tissue. A moderate plasma cellular reaction was present in this tissue and in the underlying lung. The last of the animals died before the 14th experimental day.

B. Reaction to Local Antigen Injection in Nonsensitized Rabbits

In order to determine which component of the Arthus phenomenon may be a reaction to antigen or to antibody, or to antigen-

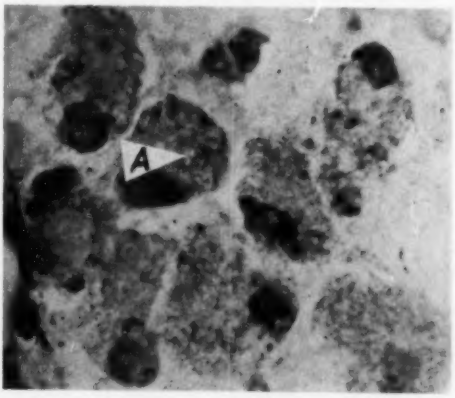

Fig. 16.—Accumulation of macrophages in the dermis at the site of antigen injection. Numerous vacuoles (*A*) and pyroninophilic granules are seen in the cytoplasm of all cells. Methyl green-pyronin; ×925.

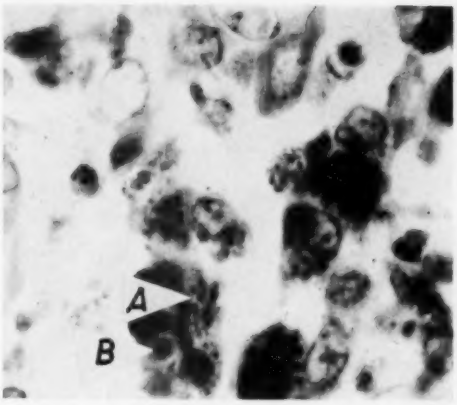

Fig. 17.—Macrophages similar to those of Figure 16, stained with PAS. The vacuoles seen in Figure 16 are PAS-positive globules (*A*), at times aggregated to form larger ones (*B*); × 925.

antibody interaction, it was decided to study the sequence of cellular and other tissue changes that occur in response to an antigen injected into a nonsensitized animal.

Material and Methods

Six New Zealand albino rabbits weighing an average of 2.8 kg. and fed Purina Rabbit Chow and water ad libidum were treated as follows: One milliliter of alum-precipitated horse serum (one part 2% aluminum potassium sulfate and two parts horse serum), was injected intracutaneously into the right flank of each rabbit on the first day of the experiment. Six rabbits were used as controls. They were given injections of 0.3 ml. of 2% aluminum potassium sulfate into their left flank and 1 ml. of alum-precipitated rabbit serum (one part 2% aluminum potassium sulfate and two parts rabbit serum) into their right flank. The horse serum had previously been Seitz-filtered, and cultures were negative for bacteria. The total protein concentration of the horse serum was 4.8 gm. %. Thus each rabbit received 32 mg. of foreign protein. The animals were killed in the following order after the injection: 24 hours, 72 hours, and 5, 8, and 14 days (two animals).

Morphological Observations

Test Sections.—Twenty-Four Hours: The antigen lay between separated collagen fibers of the dermis, intermingled with polymorphonuclear leukocytes and pseudoeosinophils. Macrophages were numerous at the periphery, but some were present also in the depth of the lesion.

Seventy-Two Hours: Extracellular antigen was present mainly in the depth of the dermis adjacent to the layer of striated muscle. Macrophages were numerous, outnumbering in most areas the acute inflamma-

Fig. 18.—Lesion of alum-precipitated horse serum; eight days. Proliferation of immature and mature plasma cells (black dots) around arteries and veins. May-Grünwald-Giemsa stain; × 70.

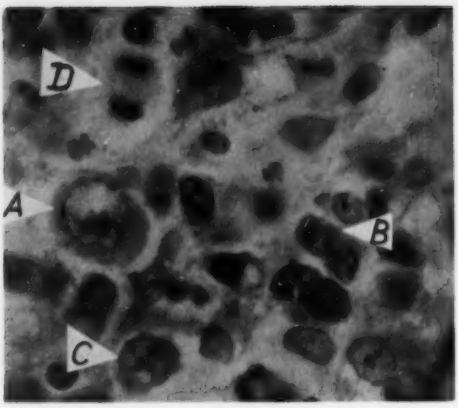

Fig. 19.—Cellular reaction in alum granuloma; eight days. Some cells are recognized as plasmoblasts (*A*); others, as mature plasma cells (*B*), and again others, as macrophages (*C*); note mitotic figure (*D*). Methyl green-pyronin; × 775.

tory cells. Lymphocytes and plasma cells could not be demonstrated in the lesion.

Five Days: The skin was about twice the thickness of normal skin. The cellular reaction was predominantly mononuclear. The antigen which was mostly in the depths of the dermis was homogeneous, staining brick red, in the routine HPS stain. In the trichrome, it stained blue with a reddish tinge. In the PTAH it did not stain like fibrin, but light brown with a purplish tinge. With PAS it stained purplish-red, and counterstaining with orange G in phosphotungstic acid did not alter this staining reaction. In the unfixed frozen sections the antigen appeared homogeneous (Fig. 15). The cells surrounding the injected antigen were breaking up and disintegrating. Although the general picture of the cellular reaction was very complex, macrophages were the predominant cells. Globular structures which were present in the cytoplasm appeared as vacuoles in MGP-stained sections (Fig. 16). Small pyroninophilic granules were distributed between these vacuoles. With HPS, trichrome, PTAH, and PAS these vacuoles appeared as globules, giving the same staining reactions as the extracellular antigen (Fig. 17). In the vicinity of vessels there was encountered a small number of large basophilic hemocytoblasts (plasmoblasts) and occasional proplasmocytes. No mature plasma cells were present.

Eight Days: The skin was still very thick. The lesion varied from the five-day one inasmuch as macrophages were more numerous and there was a fairly intense proliferation of immature and mature plasma

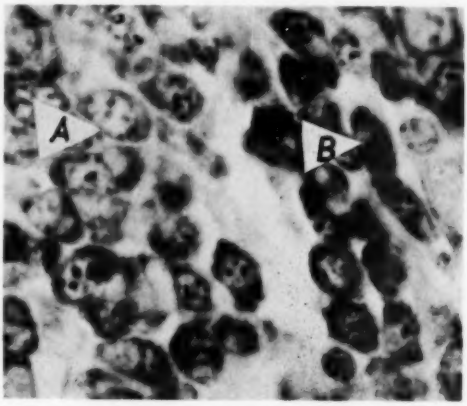

Fig. 20.—Immature (*A*) and mature (*B*) plasma cells proliferating in the dermis 14 days after a single injection of alum-precipitated horse serum. May-Grünwald-Giemsa stain; × 805.

cells. These cells were most numerous at the border between dermis and striated muscle. They showed a predilection for a perivascular distribution but occurred also diffusely in the tissues (Fig. 18). Although mature forms were already present, immature forms, i. e., plasmoblasts and proplasmocytes, were just as numerous. Mitotic figures in these cells occurred very frequently (Fig. 19). Lymphocytes were often found in association with the plasma cells.

Fourteen Days: The picture seen at this stage differed from the previous one by the abundance of mature plasma cells and by the decrease of extracellular antigen. Mature plasma cells were numerous, but immature forms were still present (Fig. 20).

Control Sections.—In the control lesions, produced by injection of aluminum potassium sulfate only, an acute inflammatory reaction was present in the 24- and 72-hour sections. From the third day on, the inflammatory reaction had an organizing character with proliferating fibroblasts and macrophages, but the latter were few in number compared with that of the horse serum lesion. No plasma cells were present in the early or late lesions. The lesion produced by injecting alum-precipitated rabbit serum also showed some proliferation of macrophages, but these were less numerous than those seen after injection of alum-precipitated horse serum. However, plasma cells were not present in the control lesions.

C. Passive Arthus Reaction

To unravel the complexity of tissue changes in relation to various immunological factors that obtain in the Arthus phenomenon, it seemed necessary to study a situation where the tissue changes as far as possible would seem to be due solely to antigen-antibody union. For this purpose the passive Arthus reaction was examined. In this state the tissues of the body are not conditioned by a previous antigen injection and there will therefore be no secondary immune response due to previous sensitization and consequently no anamnestic cellular response to confuse with the changes due to antigen-antibody reaction.

Material and Methods

Four rabbits, weighing approximately the same and being fed in the same way as those of Experiment I, received 10 ml. of antiserum intravenously per kilogram of body weight on the first day. (The antiserum was prepared in several rabbits which received repeated courses of treatment with alum-precipitated horse serum over a period of several months. Only rabbits which had a relatively high titer [1:1,000 of antibody] were bled.) Twenty-four hours later they were given 0.5 ml. of horse serum intracutaneously. They were killed 24 hours, 72 hours, 5 days, and 8 days after the local injection.

The skin with the lesion was cut into 2 mm. wide strips fixed and processed similar to the tissues in the active Arthus experiment. In addition, however, some tissues were cut fresh frozen in a cryostat instead of being embedded in paraffin.

Morphological Observations

Test Sections.—Twenty-Four Hours: The skin was swollen and microscopically the inflammatory reaction was identical to the one seen in the active Arthus reaction. However, in contrast to the lesion seen in the active Arthus phenomenon (as described in the first part of this paper), no plasmoblasts were present in the lesion.

Seventy-Two Hours: The 24-hour lesion of this rabbit differed grossly from the above 24-hour lesion by having a central hemorrhagic area which at 72 hours, when the rabbit was killed, had increased and was almost black, although the swelling had diminished somewhat.

Microscopically, in vessel walls adjacent tissue and remote from them, there was a serofibrinous exudate which widely separated muscle and collagen bundles and conglomerated in some areas into clumps and bands of a homogeneous, intensely acidophilic, and refractile material. Collagen fibers showed swelling, fragmentation, and even lysis, but there was no "fibrinoid degeneration." The cellular reaction was less marked than in the 24-hour sections, and in addition there was a striking qualitative change. Macrophages now predominated.

Five Days: At 24 hours the skin was swollen, but five days later, at the time of autopsy, the skin appeared flat, though on cross section it was somewhat thicker than the surrounding skin.

Microscopically, serofibrinous exudate and hemorrhage were no longer present. Accumulations of cells were found mainly in the vicinity of, or around, vessels. A more diffuse reaction was found in the subepidermal layer. Most of these cells were macrophages, fibroblasts, and pseudoeosinophils, but a very few polymorphonuclear cells were also present. Immature plasma cells were present in about the same number as in the lesion produced by alum-precipitated horse serum.

Eight Days: Although at 24 hours there had been swelling without hemorrhage, at the time of autopsy the skin was flat. Microscopically the lesion varied from the five-day lesion inasmuch as immature plasma cells were more numerous and some mature forms were also present.

Comment

The explosive destructive local response to injected antigen in an animal previously exposed to the same antigen is referred to as an allergic, hypersensitive, or anaphylactic tissue reaction. Some questions remain unanswered about the nature of this lesion. There is no general agreement as to whether there are any specific morphologic features that distinguish this essentially inflammatory lesion (sometimes referred to as hyperergic; Rössle [26,27]) from an ordinary acute inflammatory reaction (in contrast referred to as normergic).

The basic components of the hypersensitivity reaction, whether in the skin, pleura, or joint, as described above, consist of an acute exudative serofibrinous inflammation associated with an early proliferation of macrophages and plasma cells. This total picture differs qualitatively from a normergic inflammation,[22] and analysis of the tissue changes suggests that certain mechanisms are concerned in the pathogenesis of the different components of the lesion.

The increase of the inflammatory edema of the Arthus phenomenon in contrast to normergic inflammation has been noted by others.[12,17,24] This increase seems to be due to variations in the intensity of factors that are known to operate in the production of inflammatory edema. Factors of special significance in allergic inflammation are liberation of large amounts of histamine and thrombosis of vessels [28] but particularly spasm of vascular smooth muscle. Smooth muscle is known to be a target organ in anaphylaxis. All these and other factors undoubtedly play some role in the fact that the usual exudative components of a normergic inflammation are increased in the allergic inflammation of the Arthus phenomenon. Thus there is, so far as this component of the tissue reaction is concerned, merely a quantitative difference between the Arthus reaction, as compared to normergic inflammatory reaction. Primarily on the basis of the exudative component, Rössle [26,27] coined his concept of allergic-hyperergic inflammation which he defined as being only *quantitatively* and not *qualitatively* different from normergic inflammation.

The acute inflammation of the Arthus was identical in time and appearance to that of the passive Arthus but was absent in the alum granuloma lesion of nonsensitized rabbits. In the active and passive Arthus, where an acute exudative inflammation developed, the immunologic stage in which the tissue reaction developed included the presence of antibody in the circulation capable of reacting with the locally injected antigen.

In contrast, however, to normergic inflammation, there is in the Arthus reactions an intense proliferation of macrophages. Goddard [13] has considered this to represent a qualitatively different response from that of a normergic inflammation. Klinge,[14] Bruun,[5] and, most recently, Gell and Hinde [11] have studied the cellular components of the Arthus reaction. Gell and Hinde injected small doses of antigen subcutaneously at daily intervals and noted an early proliferation of large mononuclear

cells. On about the sixth day, they noted in addition an acute exudative inflammation similar to that of the Arthus. They considered the mononuclear cellular reaction to represent a separate component of the Arthus reaction, similar to the granulomatous response of the delayed or tuberculin type of hypersensitivity reaction. In their experiments, this mononuclear reaction occurred before the animal became sensitized. Their findings and our own could also be explained on the basis that the macrophage response represents a reaction for the removal of injected antigen. The almost certain proof of this function of macrophages in response to locally injected antigen is found in the alum granuloma lesion of our experiments, where the macrophages contained cytoplasmic vacuoles which gave all the staining reaction of the extracellular antigen. White et al.[29] and Askonas and White[1] have shown that these cells indeed contain the specific antigen which has been injected locally. Considered together these observations indicate that the macrophage response of the allergic reaction also represents the morphological counterpart of antigen removal—an opinion also expressed by White et al.[29] and Ehrich.[9] Furthermore, experiments in progress,[23] with use of immunohistochemical (fluorescent) methods, also confirm our contention that the macrophage reaction in the Arthus phenomenon represents antigen elimination.

The third component described above consisted of a proliferation of cells with basophilic cytoplasm which matured to typical adult plasma cells by the fifth day in the Arthus reaction of the skin, joint, and pleura. A plasma cellular reaction was not described by Klinge[14,15] in his studies of the local hypersensitivity reaction of the rabbit joint. Others[5,11,13] have described plasma cells in the Arthus reaction, but they have not looked upon this finding as a unique morphologic feature that brings some morphological specificity to the Arthus phenomenon. Plasma cells are known to produce antibodies,[4,6-8,10,18,20,25] and these cells are not known to have any other function. It is therefore a reasonable assumption that their presence indicates local formation of antibody. Their presence in the Arthus reaction is strong evidence for the formation of antibodies at the site of local antigen injection. That they are not part of the acute anaphylactic inflammation is attested by their development in the alum granuloma where no acute inflammation occurred. It is also interesting that the appearance of plasma cells six days after a local injection into the nonsensitized rabbits coincides in time with the proliferation of plasma cells in lymph nodes and spleen after a single intravenous injection of antigen. The finding of plasma cells in the alum granuloma is at variance with White and associates,[29] who found plasma cells only in the regional lymph nodes. Dosage and the type of antigen may well account for these differences. The formation of plasma cells in four to five days in the Arthus we believe is the morphological expression of a *secondary immune response*, whereas their appearance in six days in the passive Arthus and alum granuloma are the morphologic counterpart of a *primary immune response*.

The analysis of the foregoing observations indicates that the morphological features of the Arthus phenomenon represent a lesion pattern that is highly characteristic for an actively induced hypersensitive response of tissues. This reaction consists of an acute serous and serofibrinous exudate and an accumulation of a large number of mononuclear cells (macrophages and plasma cells). Together these constitute the *qualitative* property that differentiates the Arthus reaction from a normergic inflammation. We are inclined to consider the acute exudative and macrophage components as representing an *allergic inflammation*. The evidence presented supports the hypothesis that the exudation of this allergic inflammation is the result of an *antigen-antibody reaction*, whereas the macrophage response represents *antigen removal*. The third component, the plasma cellular reaction, we believe, represents *antibody production* and

accordingly is not part of the inflammatory reaction but results from antigenic stimulation of primitive mesenchymal cells. On this basis, the allergic inflammation, together with the plasma cellular reaction, constitutes the morphological counterpart of a local immediate or anaphylactic type of hypersensitivity reaction.[22] The active Arthus phenomenon is the prototype of such a reaction.

Summary and Conclusions

The morphology of the active and passive Arthus phenomena is described, as well as the changes in the dermis following the injection of antigen into a nonsensitized rabbit.

From these studies it was concluded that the tissue reaction of the active Arthus phenomenon differs from a normergic inflammatory reaction by the simultaneous development of three components: serous or serofibrinous exudation, intense proliferation of macrophages, and maturation and proliferation of plasma cells. The morphological hallmark of the lesion is the presence of an intense mononuclear (macrophage and plasma cellular) response that is out of proportion to that expected in what is otherwise an acute exudative inflammation.

Pathogenetically, the exudative component is believed to be initiated by marked circulatory disturbances, which are primarily the result of smooth muscle damage in anaphylaxis. The exudative changes progress further following vascular leukocytic, platelet, and fibrin thrombosis. This component of the lesion results following antigen-antibody *interaction.* The macrophage reaction is thought to represent the *morphological counterpart of antigen removal.* The third and most important component in differentiating the allergic tissue reaction from the normergic inflammation is the proliferation of plasma cells. These are considered to be the *morphological counterpart of antibody formation.*

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Experimental Meningoencephalomyelitis Produced by Hemophilus Pertussis

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The rare, but impressive, occurrence of encephalomyelitis in the course of pertussis and of encephalopathy following injection of pertussis vaccine is well known. Since many accept hypersensitivity as the most likely explanation of these complications, and since Hemophilus pertussis possesses certain allergenic attributes, the present study was made.

The most effective method of producing experimental encephalomyelitis is the injection of a mixture of brain or spinal cord with a combined adjuvant consisting of paraffin oil, an emulsifying agent, and killed Mycobacterium tuberculosis.[1,2] This procedure is based upon the theory that the resulting lesion in the central nervous system is the result of an antigen-antibody reaction. According to this hypothesis the important factor in the pathogenesis of this experimental disease is the production of a delayed type of induced sensitivity known as the "Dienes effect." Dienes[3,4] was able to produce in the guinea pig both immediate and delayed sensitivity to various proteins (egg-white albumen, horse serum, pollen, etc.) by injecting these antigens into a local lesion previously produced by the injection of either living or killed Mycobacterium. The presence of Mycobacterium enhanced the total antibody produced to the injected protein. It was later learned that paraffin oil also enhances the antigenicity of killed Mycobacterium. Freund and McDermott[5] utilized the Dienes effect plus the adjuvant effect of oil to produce a severe delayed type of animal skin sensitivity to injected horse serum. This was done by injecting oil and Mycobacterium (the Freund antigen) and horse serum into the same site.

Kabat, Wolf, and Bezer[1] produced encephalomyelitis in 17 of 19 rhesus monkeys with use of an emulsion antigen composed of paraffin oil, Aquaphor (an emulsifying agent), adult rabbit or monkey brain, and killed tubercle bacilli. Each milliliter of the final emulsion was equivalent to from 175 to 225 mg. of original tissue and 0.5 mg. of dried killed tubercle bacilli. The final concentration of phenol was 0.25%. Freund, Stern, and Pisani[6] successfully applied the adjuvant technique in the guinea pig.

Many variations concerning the antigen, experimental animal, and method of injection have been tested. Practically all of the adjuvant antigens reported have contained M. tuberculosis, other acid-fast bacilli, or their fractions.

In the experiment here reported killed H. pertussis was substituted for killed M. tuberculosis in the antigen.

Method and Materials

Animal.—The Hartley strain of guinea pig was used because it has been reported[7] to be highly susceptible to other forms of allergic encephalomyelitis. Fifty female pigs weighing from 350 to 450 gm. each were assigned to five groups of ten each.

They were kept in separate cages and allowed food and water without restriction. They were observed daily during the first 10 days and thereafter two or three times daily. Daily temperatures, weights, and neurological signs were recorded.

All surviving animals were killed during a 4-day period 18 to 21 days after injection of the antigen. For every animal in the experimental group, a

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control (from Groups A, B, and C) was killed simultaneously.

Antigen.—This consisted of rabbit spinal cord previously shown capable of causing encephalomyelitis in the guinea pig in a dose of 0.08 mg. when injected as a part of the Freund antigen. It was stored at —5 C until used three days later.

Five separate materials for injection were prepared; one for each group of 10 animals. These five groups were designated Groups A, B, C, D, and E.

Group A (control, phenol alone): A 0.5% phenol solution was prepared by diluting 5 cc. of 1% phenol to 10 cc. of distilled water.

Group B (control, dried killed H. pertussis, Bayol F, and Arlacel A)*: An oil suspension of dried killed H. pertussis was prepared with a concentration of 10 mg. per milliliter by mixing 8.5 cc. of Bayol F with 1.5 cc. of Arlacel A and adding 100 mg. of dried killed H. pertussis. The mixture was autoclaved.

Group C (control, rabbit spinal cord in 0.5% phenol plus Bayol F and Arlacel A).

Group D (experimental): Rabbit spinal cord in 0.5% phenol plus Bayol F and Arlacel A plus H. pertussis. H. pertussis was added to the cord mixture in a concentration of 20 mg. of dry killed organisms per milliliter. The final concentration was 10 mg. per milliliter.

Group E (positive control): Rabbit spinal cord in 0.5% phenol plus Bayol F and Arlacel A* plus dried killed M. tuberculosis. The concentration of M. tuberculosis was 12 mg. per milliliter.

Preparation of Material for Pathological Examination.—With the animal under ether anesthesia, the pleural cavity was exposed by removing the sternum. After 5 to 10 cc. of blood was obtained from the heart the left ventricle was opened near the apex, through which a thin glass cannula was inserted into the ascending aorta and tied in place.

Saline was perfused until the perfusate ran clear. This was followed by a 15% formalin solution under a pressure of 60 cm. of water. The animal was preserved in formalin for 10-16 days, after which the brain and cord were removed for further examination. Microscopic sections were stained with hematoxylin and eosin and with Lillie's variant of the Weil-Weigert stain for myelin.

* Bayol F is a paraffin oil of low viscosity (Stanco Distributors, N. Y.). Arlacel A (mannide monmooleate) is a nonionic emulsifying agent (Atlas Powder Company, Wilmington, Del).

Each of the 10 animals in Groups B, C, D, and E received two simultaneous intradermal injections on the lower back of 0.1 cc. each. Group A animals received only 0.1 cc. of 0.5% phenol.

Below are listed the amounts of material injected:

Group	Penol, Mg.	H. Pertussis, Mg.	M. Tuberculosis, Mg.	Rabbit Cord, Mg.	Bayol F + Arlacel A, Cc.
A	0.5				
B		2			0.2
C	0.5			11	0.1
D	0.5	2		10	0.1
E	0.5		1.3	12	0.1

Results

The animals in Groups A, B, and C gained weight after injection, reaching a maximum on the day they were killed. The average weight gains in grams were Group A, 73.7; Group B, 61.5; Group C, 57.7. None of these animals revealed neurological signs, and no pathological lesions were revealed by examination of brain or cord.

In Group D the animals gained normally until the 10th to 12th days. During the 11th and 12th days Animal D-5 lost 108 gm. and developed fever and neurological signs. Between Days 12 and 13 all animals in Group D except D-2 began to lose weight and continued to do so for the next six to nine days. In D-7 and D-8 weight loss preceded rise in body temperature. The average weight loss in this group was 63 gm.

Neurological.—In Group D (H. pertussis adjuvant) the following abnormalities were noted.

D-5 (Fig. 1): This animal developed a temperature of 40 C on the 12th day after injection. Tachycardia was noted. It appeared ill, failed to feed, and had a ruffled coat. In two days it lost 108 gm. in weight. General body tremor and rear limb weakness were noted. On the 13th day the spine was flexed and the head extended. The

Fig. 1.—Paralyzed, incontinent guinea pig 11 days after injection with adjuvant antigen containing H. pertussis.

extremities were flexed, and rapid myoclonic convulsions occurred. There was spastic paralysis of the extensor muscles of the legs. The animal moved by dragging its rear limbs and by making scratching movements with its front feet.

D-6: On the 13th day this animal showed rear limb weakness, decreased abdominal muscle tone, and an abnormal righting reflex. Weakness progressed during the next six days to involve the left anterior limb.

D-10: Transient rear limb weakness and abnormal righting reflex were noted on the 13th day.

The remaining animals in Group D did not exhibit any localized neurological signs.

All except D-2 and D-3 were febrile, and all except D-2 showed weight loss. All moved slowly and appeared weak.

Pathological Findings.—All animals in Group D except D-2 and D-3 manifested microscopic evidence of meningoencephalitis. The commonest sites were the meninges, the subpial and subependymal blood vessels, the tela choroidea, and the choroid plexus of the third and fourth ventricles. Paraventricular lesions were common. Lymphocytes, histiocytes, and some monocytes were the usual infiltrating cells, although neutrophils were occasionally noted. Proliferation of glial cells in the perivascular space was common.

The most frequent pathological findings were lymphocytic perivascular infiltration, meningitis (Figs. 2, 3) and choroiditis. Ependymitis and glial proliferation were also frequently observed (Fig. 4).

In the cord satellitosis and neuronophagia were occasionally observed, but nerve-cell degeneration was not a prominent finding. In some areas the perivascular cuffing extended into and involved the adjacent

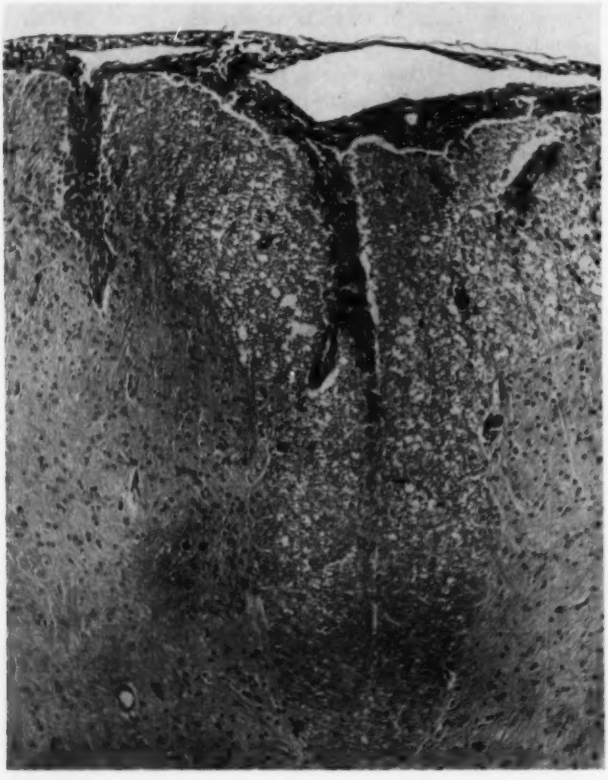

Fig. 2.—Section of spinal cord from Animal D-9, showing lymphocytic meningitis and perivascular infiltration. Hematoxylin and eosin; × 100.

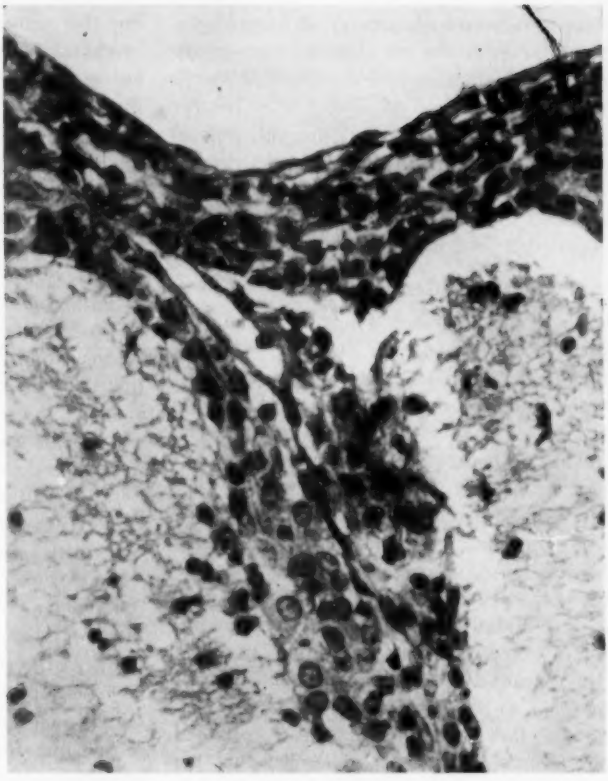

Fig. 3.—Higher power of Figure 2, showing lymphocytic reaction. Hematoxylin and eosin; × 900.

parenchyma (Fig. 4). With the exception of a few suspicious perivascular areas, little or no demyelination was observed.

In Group E (M. tuberculosis adjuvant) all of the animals lost weight after the 10th to 12th days, which coincided with rise in

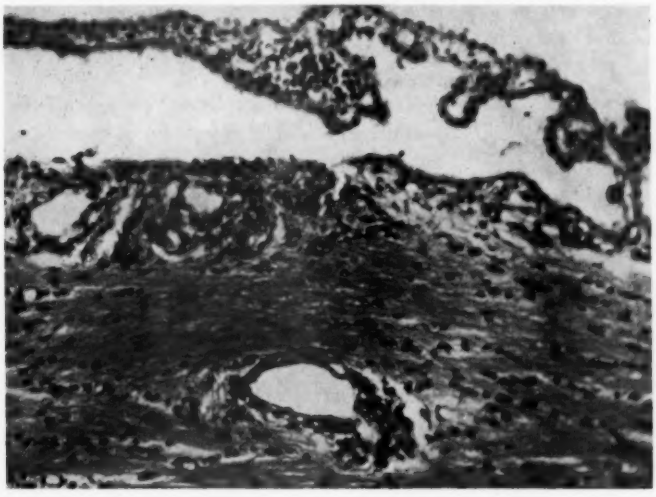

Fig. 4.—Choroid plexis from Animal D-9, showing cellular infiltration with severe involvement of adjacent parenchyma with perivascular cuffing and subependymal infiltration. Hematoxylin and eosin; reduced about 20% from mag. × 250.

temperature and appearance of neurological signs. Seven of the ten animals in this group died within four days after onset of symptoms. The course of illness was severer among these animals, and the pathological findings, though similar to those observed in the animals of Group D, were more pronounced. All of the animals in this group revealed lesions in the brain and cord.

Comment

In this experiment 8 of the 10 animals given injections of the antigen containing killed H. pertussis revealed evidence of pathological changes in the central nervous system. In five of the eight showing such lesions no neurological signs were observed during life. In one a fulminating encephalopathy resulted.

Neither neurological nor pathological findings were so severe in the pertussis as in the tuberculosis group, though the pathological findings were similar. Cellular infiltrates were prominent, while neuronal damage and demyelination were insignificant findings. A possible explanation of this may be the fact that the animals were killed too soon. Moreover, it is known that the guinea pig does not reveal demyelination as a prominent feature of experimental encephalomyelitis so readily as do other species.[6,8]

Although the mechanism involved in the production of the experimental lesion has received much attention, the role played by M. tuberculosis and, in this experiment, by H. pertussis in the adjuvant antigen concerns us most. In recent studies reported M. tuberculosis has been used. Insofar as we are aware, this is the first report of the use of H. pertussis in an attempt to produce the lesion.

From studies of the experimental disease in the animal it is generally agreed that the successful production of the lesion depends upon the development by the animal given an injection of a tuberculin type of sensitivity to some component of nervous tissue. There is evidence that the effect of the tubercle bacillus in the adjuvant in producing this sensitivity is due to its lipopolysaccharide content. In fact, this fraction can successfully replace the whole organism in the adjuvant.[9]

For some time the sensitizing nature of H. pertussis has been known. Toomey[10] emphasized allergy as an important factor in the mechanism of infection caused by this organism. Parfentjev[11] demonstrated that laboratory animals (mice and guinea pigs) given injections of H. pertussis antigens developed sensitivity to the nucleoprotein complex of the organism. Pittman[12] found that mice infected intranasally with suspensions of the organism exhibited a high degree of sensitivity to histamine. Sensitized mice subsequently given injections of a sonic extract of the organism develop a much greater degree of leukocytosis than do normal mice, particularly in regard to the granulocytes.[13] It is also reported that the lipid content of H. pertussis is much higher than of other commonly encountered pathogens of the respiratory tract, with the possible exception of the tubercle bacillus. While none of these facts may be directly responsible for the organism's part as an adjuvant antigen in the lesions described here, it is interesting to speculate as to their possible role. Experiments designed to study the effect of chemical fractions of the organism are planned.

Summary

Experimental encephalomyelitis has been produced in the Hartley strain of guinea pig by the intracutaneous injection of an adjuvant antigen containing rabbit spinal cord, paraffin oil, an emulsifying agent, and killed Hemophilus pertussis.

The lesions produced were predominately meningeal and perivascular and, though less severe, resembled those produced by a similar antigen containing killed Mycobacterium tuberculosis.

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News and Comment

PERSONAL

Dr. R. Winston Evans at University of Chicago.—Dr. R. Winston Evans, of the Department of Clinical Pathology at the University of Liverpool, is serving as visiting professor of pathology at the University of Chicago during the winter quarter, 1959.

Award to Dr. F. K. Mostofi.—Dr. F. K. Mostofi, scientific director of the American Registry, Armed Forces Institute of Pathology, was recently awarded a Letter of Appreciation from the Secretary of Defense for his contributions to the Joint Committee on Aviation Pathology.

Markle Scholar Appointments for Drs. Barry Pierce and Huntington Sheldon.—Among this year's appointments as Markle Scholars in Medical Science by the John and Mary R. Markle Foundation, New York, are two pathologists. These are Dr. Barry Pierce, Assistant Professor of Pathology in the University of Pittsburgh School of Medicine, and Dr. Huntington Sheldon, Assistant Professor of Pathology in McGill University Faculty of Medicine.

ANNOUNCEMENTS

American Institute of Dental Medicine.—The 16th Annual Meeting of the American Institute of Dental Medicine will be held in Palm Springs, Calif., Nov. 8-12, 1959. Further details may be obtained from the American Institute of Dental Medicine, 2240 Channing Way, Berkeley 4, Calif.

Henry Ford Hospital Symposium on Pyelonephritis.—The Henry Ford Hospital International Symposium on "The Biology of Pyelonephritis" will be held at the Henry Ford Hospital, in Detroit, on Oct. 8, 9, and 10, 1959. Further information may be obtained from Dr. E. L. Quinn, Program Committee Chairman, W. Grand Blvd. at Hamilton, Detroit 2.

Sixth International Congress for Internal Medicine.—The Sixth International Congress for Internal Medicine will be held in Basel, Switzerland, Aug. 24-27, 1960. For further details, apply to the Secretariat of the Sixth International Congress for Internal Medicine, 13, Steinentorstr., Basel.

GENERAL NEWS

Films.—The following is a list of films of possible interest to pathologists.

Title: "Cytological Aspects of Immunohematology"; 15 minutes, silent, black and white, 195—.
Producer and Author: Marcel C. Bessis, M.D., Centre National de Transfusion Sanguine, Paris.
Distribution (in U. S.): The International Society of Hematology, Office of Secretary-General, Western Hemisphere, Suite 6D, 1180 Beacon St., Brookline 46, Mass.
(Note: Narration is recorded in *Annals of the New York Academy of Sciences,* Vol. 59, Article 5, under the title "Leucocytic Functions.")

Title: "Hormonal Cyto-Diagnosis: Vaginal Smears"; 21 minutes, color, sound, 16 mm., 1955.
Director and Producer: This film was produced under direction of Prof. agr. André Sicard (Paris) by Dr. Raoul Godet, assisted by Dr. M. Pariente, and Dr. G. Marsan, and with the collaboration of Ciba Limited.
Distribution: Film Library, American Medical Association, 535 N. Dearborn St., Chicago 10.

Title: "Routine Pelvic Examination and Cytologic Method"; 14 minutes, sound, color, 1958.
Professional Consultant: S. B. Ginsberg.
Sponsors: American Cancer Society and National Cancer Institute, National Institutes of Health, U. S. Department of Health, Education, and Welfare.
Producer: Audio Productions, Inc., New York.
Distribution: American Cancer Society, Professional Film Library, 521 W. 57th St., New York 19, and its divisions.

Title: "Handling Laboratory Animals"; 20 minutes, sound, black and white, 16 mm.
Producer: MacQueen Films, Ltd., in cooperation with the National Institute of Medical Research, England.
Distribution: Animal Welfare Institute, 22 E. 17th St., New York 3.
Cost: Purchase, $65 (no charge for preview); one week rental, $3.

Title: "Technique of Proctoscopy"; 17 minutes, sound, color, 16 mm., 1956.
Authors: Drs. Raymond J. Jackman and Edward R. Morgan.
Producer: Mayo Clinic, Rochester, Minn.
Distribution: Film Library, American Medical Association, 535 N. Dearborn St., Chicago, 10.

A REQUEST FOR CERTAIN AUTOPSY MATERIAL TO FURTHER NORMATIVE RESEARCH ON THE MYELINATION OF THE HUMAN NERVOUS SYSTEM

The undersigned are currently engaged in a study of the standard criteria of the normal myelination of the human nervous system from birth to puberty. The purpose of the study is to establish comparative age gradients of normal myelination for comparison with the retarded and pathological myelination, employing routine histological techniques in common usage (Heidenhain, Weil, Loyez, and Klüver-Barrera methods) for staining myelin.

The autopsy material of reasonably normal cerebra and spinal cords of human infants and children is difficult to collect from any single geographic area of the population in a statistically sufficient number and age distribution from birth to puberty.

Through the medium of this journal we appeal to the departments of pathology and to medical institutions authorized to perform autopsies to make available to us such pertinent cerebra and spinal cords of infants and children up to puberty as they may be willing to entrust to us for study and may deem practicable.

The specimens of cerebra of infants and children should be reasonably free from disease processes which caused death of the infant or child. The cerebra should be removed whole with the brain stem and cerebellum and should perferably be perfused immediately after removal from the cranium with 200-300 cc. of the usual 4% to 10% formalin solution through the internal carotid and vertebral arteries. The so-perfused specimens should be suspended in at least ten times their volumes of formalin for a few days to two weeks. When reasonably fixed, they should be placed in a cellophane bag and packed with ordinary vaseline in a tin can of suitable size. The tin cans and packing material will be supplied on request. The sealed tin can with the specimen and information on it should be sent to Railway Express, C. O. D. to:

Myelination Laboratory
Warren Anatomical Museum
Harvard Medical School
25 Shattuck St., Boston 15

Specimens so received by us will be prepared in gapless serial whole-brain celloidin sections. The investigators agree to supply the sender of each pertinent specimen with a complete set of unstained sections at intervals of every 20th section through the entire specimen. The specimens which should be found by us not suitable for purposes of this study will be returned to the sender at our cost.

All expenses entailed in preparing, packing, and shipping the specimens will be met by the Myelination Laboratory.

PAUL I. YAKOVLEV, M.D.
EDWARD P. RICHARDSON, M.D.
Warren Anatomical Museum
Harvard Medical School

Books

Dorland's Pocket Medical Dictionary. Twentieth edition; abridged from "Dorland's Illustrated Medical Dictionary." Edited by L. B. Arey et al. Price, $4.50. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1959.

This little dictionary has been rechristened "Dorland's Pocket Medical Dictionary" in honor of Dr. W. A. Newman Dorland. Its purpose, for many years, has been to give the latest in medical terminology "in a compact and convenient format." The present editors have made every effort to keep abreast of the changing emphasis and increasing knowledge in medicine and the related sciences. Emphasis is placed on pronunciation and on brevity. The book should continue to serve as a useful adjunct to the larger "Dorland's Illustrated Medical Dictionary," the book from which this one is derived.

Viral and Rickettsial Infections of Man. Third edition. By Thomas M. Rivers, M.D., and Frank L. Horsfall Jr., M.D. Price, $8.50. Pp. 967, with illustrations. J. B. Lippincott Company, 227-231 S. 6th St., Philadelphia 5, 1959.

In the third edition of this masterly book the forty-four contributors have attempted to bring up to date the vast amount of information which has become available in the past six years. Each contributor is eminent in this field; the result is a source book of exceptional value to every one interested in viral and rickettsial disease, whether student, teacher, or investigator. Owing to generous financial support from the National Foundation, the book is made available at an exceptionally low cost. It will continue to be of immense value to all students of disease.

The Physiology and Pathology of the Cerebellum. By Robert S. Dow, M.D., and Giuseppe Moruzzi, M.D. Price, $12.50. Pp. 675, with 182 illustrations. University of Minnesota Press, Minneapolis 14, 1958.

This book is divided into two major sections: "Part I, Physiology," and "Part II, Clinical Symptomatology and Pathology," and is intended to supplement a forthcoming book on the anatomy of the cerebellum by Dr. Olof Larsell. This accounts for the obvious paucity of anatomical information in the text.

It contains a detailed consideration of cerebellar physiology which is authoritative and complete, and it is this feature that represents its greatest value. This information is succinctly summarized in Chapter 7. The clinical and neuropathological section of the book is of more general interest and covers the wide range of various neurological diseases as they affect the cerebellum. The neuropathology is largely descriptive and to a certain extent is handicapped by lack of clarity in the halftone illustrations. The bibliography is extensive, and the book is clearly a fund of information about the cerebellum.

Action of Radiation on Tissues: An Introduction to Radiotherapy. By A. Lacassagne and G. Gricouroff. Translated by Clarence C. Lushbaugh, M.D., and Gretchen R. Riese, M.S. Price, $6.25. Pp. 199, with 17 illustrations. Grune & Stratton, Inc., 381 4th Ave., New York 16, 1958.

When a scientist chooses to translate a scientific classic he performs a real service for his confreres. It is unfortunate that scientific erudition has often been sacrificed for scientific specialization. Thus this classic work in the field of radiation effects, although known by reputation, has largely been lost to investigators in this country. Lacassagne has had a long and distinguished career in the field of radiation and is today widely recognized as a foremost authority on tissue effects of radiation. The original volume, published in 1941, was addressed to young physicians preparing for a specialty demanding radiobiologic knowledge. Following World War II the text was extensively revised to include recent advances in radiobiology and the extensive use of radioisotopes.

The monograph first considers the biological actions of radiations, explaining the modes of action of ionization radiation and the degeneration induced in tissues by such radiation. Lacassagne then methodically reviews radiation effects in various organ systems, concluding with brief considerations of total-body radiation and the effects of radiation on neoplastic tissues. In each of the sections both gross, visible changes in the organ and the histologic

alterations are discussed. Fortunately, in most areas the tissue changes are well coordinate with radiation dose.

The monograph makes no pretense of completeness. Its purpose is to survey briefly radiation effects in the various tissue, and, since it is primarily intended for physicians, the effects in human tissues are primarily presented. Supplementation with experimental data in various species is used as necessary. What the volume lacks due to brevity, it compensates for with its bibliography. All of the important references appear to have been included in each of the chapters, and these have been further supplemented by the translators, who have thoughtfully included recent, particularly American, references. The emphasis on pathologic effects of ionizing radiation make this volume essential for the pathologist who must deal with radiated tissues, particularly if he is not already well acquainted with these tissue effects. In addition anyone dealing with radiation, either experimentally or therapeutically, will find this book most useful. Dr. Lushbaugh, a distinguished investigator in radiation tissue effects, has done a real service in making this volume readily accessible to American medicine.

Standard Methods of Clinical Chemistry. Vol. II. By the American Association of Clinical Chemists; David Seligson, Editor-in-Chief. Price, $5.50. Pp. 224, with 4 illustrations. Academic Press, Inc., 111 5th Ave., New York 3, 1958.

Among the foremost objectives of the American Association of Clinical Chemists is the standardization and codification of methods, and this volume, the second in a series, is a significant contribution. It presents twenty-one individual methods, in each case delineates the underlying principle, describes precisely the preparation of reagents and sequence of procedures, and gives the range of normal and pathological values.

The subject matter, arranged alphabetically rather than systematically, is a rather arbitrary cross section of current activity in this field, by fifty-eight authors and checkers from twenty-two states and one foreign country. Described are newer (usually more rapid and convenient) procedures for old-time substances like calcium, chloride, and cholesterol; also methods for new candidates for the routine laboratory—γ-globulin, 17-hydroxycorticosteroids, iron in serum, 17-ketosteroids, porphyrins, and protein-bound iodine; some classic analyses—nitrogen by the Kieldahl method, hemoglobin, and nonprotein nitrogen. Included also are methods for testing of cephalin-cholesterol flocculation, sulfobromophthalein, urobilinogen, phosphatase, lipase, and phosphatides.

The style is direct and aimed to give the maximum practical information to the laboratory technician. Two topics, blood pH and flame photometry, are treated at greater length with more extensive discussion of principles and alternative procedures. Except for these last two topics, each method was checked by one or more independent investigators in other laboratories, and this has served to insure practicability and to eliminate errors.

Diagnostic Laboratory Hematology. By George E. Cartwright, M.D. Price, $6.75. Pp. 250, with 33 illustrations. Grune & Stratton, Inc., 381 4th Ave., New York 16, 1958.

The first edition of this small laboratory manual was intended to be a manual for students and busy physicians and, as such, was of limited scope and included only simple diagnostic procedures requiring a minimum of equipment. Apparently, the limitation became apparent and the second edition was therefore considerably enlarged. It now includes methods for virtually every diagnostic procedure used in Hematology, with the possible exception of the absence of methods for determining total blood volume and red cell survival. But these are certainly not simple and routine studies which are commonly performed, and so this omission is not a serious handicap. Some will object that this is purely a "cook book," but, in this reviewers opinion, it is a good "cook book." The techniques are well outlined, and the instructions include even minor details. In some of the newer procedures a brief historical and theoretical background is given, but generally the theoretical basis of the various procedures is not adequately considered. For most of the procedures references to the literature are given so that more detailed information may be obtained, if desired. All in all, this book will serve very adequately in the capacity for which it is intended.

Tumors of the Soft Somatic Tissues. By George T. Pack, M.D., L.L.D., and Irving M. Ariel, M.D., with 15 collaborating authors. Price, $30. Pp. 820, with 468 illustrations and 109 tables. Paul B. Hoeber, Inc. (medical book department of Harper & Brothers), 49 E. 33d St., New York 16, 1958.

Tumors of soft somatic tissues present some of the more difficult problems that surgeons and pathologists alike must face. Drs. Pack and Ariel have recognized the lack of good reference

works in this field and have attempted, in this volume, to present a comprehensive account of soft tissue tumors. The volume is divided into six sections, opening with a brief consideration of classification and natural history of soft tissue tumors. A discussion of general principles of treatment is followed by a section on treatment of specific tumors, which occupies well over half of the book. The final hundred pages are devoted to sarcomas in children, regional anatomic considerations in treatment, and prognosis.

Perhaps, since the book is written by surgeons for surgeons, pathologists should not be overly critical. While there can be little quarrel with the authors' statements that benign tumors seldom become malignant and that rarely does trauma appear to play a significant role in tumor development, there may be some question about the apparent emphasis put on the occasional phenomenon of lymphatic metastases of sarcomas in the first sections. It is in the organization of the chapters on specific tumors that pathologists will find the most confusion. The inclusion of Dupuytren's contracture, Peyronie's disease, carcinosarcoma of the uterus, and Wilms' tumor in the chapter on fibrous tissue tumors will shock many pathologists.

However, if he will overlook such idiosyncrasies of classification, the pathologist will find some things that are of interest to him, particularly when he consults with surgeons. The senior author has had a distinguished career in tumor surgery at the most active cancer center in this country. The volume has the benefit of his twenty-five years of experience. While it is advantageous to draw on one surgical group's experience for a discussion of soft tissue tumors, it is also something of a liability to be restricted to a single group's experience for a consideration of such a comprehensive subject. It is this restriction of viewpoint that is the most serious criticism of this volume. Often the illustrative case reports seem to be selected from the authors' series on the basis of particularly good surgical results rather than whether the case exemplified the tumor in its classical concepts. References are often outdated and appear weighted with the authors' previous reports. The works of others are used only in the general discussions of the various tumors rather than in regard to treatment and prognosis and even then are handled in a confusing manner. If he wants a serious, definitive, well-documented discussion of soft tissue tumors, the pathologist will be disappointed in this book. If an occasionally superficial, always interesting, surgically oriented consideration of such tumors will do, this is his book.

Advance in Veterinary Science. Vol. III. Edited by C. A. Brandly and E. L. Jungherr. Price, $13. Pp. 579. Academic Press, Inc., 111 5th Ave., New York 3, 1957.

Most sections of this volume are written primarily for veterinarians. However, general pathologists, neuropathologists, and neurologists, as well as students of comparative disease, will be interested in the large section (163 pages) on "Diseases of the Central Nervous System of Domesticated Animals and Comparisons with Human Neuropathology." This title is somewhat misleading, since human neuropathology is considered only slightly. Pathology is emphasized, but etiology, course of the disease, and clinical findings are also considered. The authors indicate that this material will be expanded into book form and subjects not covered here such as neuropathology of laboratory animals, diseases of the hypophysis, spinal cord, and skeletal muscle will be included. Until the book is published, this rather comprehensive review of animal neuropathology is a valuable source of information.

"Anesthesiology," "Eradication of Bovine Brucellosis in Scandinavia," "Brucellosis in Goats and Sheep," "Swine Brucellosis," "Hemminthic Diseases," "Antinematodal Drugs," "Artifical Insemination of Cattle," and "Clostridial Diseases of Animals" are the titles of the other reviews in this volume.

Clinical Endocrinology. Second edition. By Karl E. Paschkis, M.D.; Abraham E. Rakoff, M.D., and Abraham Cantarow, M.D. Price, $18. Pp. 941, with 274 illustrations, 6 in full color. Paul B. Hoeber, Inc. (medical book department of Harper & Brothers), 49 E. 33d St., New York 16, 1958.

Written as an outgrowth of presentation, over the years, of endocrinology to undergraduate and graduate students and practitioners, this book was organized to attempt to meet the needs of these groups. Material is presented for each organ in the sequence of embryology, anatomy, histology, physiology, pathology, pathologic physiology, diagnosis, and treatment. When possible, the hyperfunctioning and hypofunctioning disease states are considered separately. This format was chosen so that the undergraduate student can find a text to be read in consecutive order and the practioner can find approaches to diagnosis and therapy. While this organization fulfills these objectives rather well, it does tend to compartmentalize the presentation, to inter-

rupt continuity of thought, and to result in a certain amount of repetition. However, it also facilitates the use of this book as a reference.

The second edition incorporates well the recent developments. Sections on thyroid and adrenal have been expanded and revised the most. The bibliography includes many review articles.

Steroid Homeostasis, Hypophysis and Tumorigenesis. By Alexander Lipschutz. Price, 15 shillings. Pp. 92. W. Heffer & Sons, Ltd., Cambridge, England, 1957.

This is an up-to-date enlargement of Professor Lipschutz' address at the Sixth International Cancer Congress in São Paulo, Brazil, in 1954. It provides an extremely valuable and critical review of recent developments in experimental cancer endocrinology and at the same time summarizes the important discoveries and views of the distinguished author. Professor Lipschutz marshals pertinent evidence that steroid hormones—especially estrogens—not only are concerned with the induction of tumors but that they may also suppress tumor formation. He cites convincing experiments which show that the endogenous steroids of the host may support or induce cancerous growths. In large measure, this dispells doubts as to the general validity of experimental results with tumors produced by administered steroids.

The leading concept developed in this book concerns the causal relation between the production of certain tumors and disturbances of the endocrine "constellation" of the host. Lipschutz espouses the view that imbalances between the trophic hormones of the pituitary and the steroid hormones of the ovary are responsible for the development of several experimental neoplasms. These imbalances of steroid homeostasis may originate not only from primary ovarian or hypophyseal failure but likewise from disturbances in the cyclic nature of ovarian function. Steroid homeostasis, according to the author, is essential for the maintenance of the nontumorous condition of the body, for it provides what he terms "antitumoral autodefence."

There is much wisdom in the views expressed in many areas of this monograph, and there are several interesting suggestions for the further development of cancer endocrinology. Professor Lipschutz writes, one imagines, in very much the same style as he speaks. The English language in many places is somewhat unidiomatic, and although this calls for reconsideration of passages by the reader, it only adds to the personal and intimate charm of this little book.

Medical Electrical Equipment: Principles, Installation, Operation and Maintenance of Electrical Equipment Used in Hospitals and Clinics. By Robert E. Molloy, M.B., F.F.A., R.C.S. Price, $15. Pp. 320, with 238 illustrations. Philosophical Library, Inc., 15 E. 40th St., New York 16, 1958.

Authoritative information on the principles, operation, care, and maintenance of electrical equipment currently used in medicine and surgery is presented by twenty-two authors, chiefly engineers of various British instrument-manufacturing companies and chief technicians in leading English hospitals. Chapter headings include illumination, refrigeration and air conditioning, x-ray, radioisotope instruments, infrared and ultraviolet lamps, endoscopes, electrocardiograph, electroencephalograph, artificial respiration, spirometry, photographic dark rooms, dental engines, bone saws, incubators, water baths, sterilization equipment, ovens, incinerators, and conveyors. There are also general chapters on the nature of electric currents, wiring, transformers, methods of discharging static electricity, small electric motors, and testing and maintenance of electrical devices.

The style is simple and direct, with a fine balance between basic principles and definition of terms on the one hand and practical operating instructions, safety precautions, trouble-shooting, servicing and maintenance, and applications on the other. The voltage in Britain is standardized at 240, and the frequency of alternating current is 50 cycles per second, but this in no way decreases the value of the book for Americans.

The Middle Ear. By Heinrich G. Kobrak. Price, $15. Pp. 254, with 41 illustrations. The University of Chicago Press, 58th St. & Ellis Ave., Chicago 37, 1959.

This book presents current information on the diagnosis and treatment of deafness due to disease of the conduction apparatus. The author introduces the subject by a thorough analysis of the anatomy and physiology of this system and further along presents pathological material including many colored photographs to illustrate the many kinds of middle ear disease that produce hearing loss. Dr. Kobrak was widely known for his researches in this field.

Six specialists contribute chapters, each in his own particular area of competence. A chapter on methods of testing auditory function by Fournier is followed by a chapter on the fenestration operation by Lindsay and one on the stapes mobilization operation by Rosen. Chapters on tympanoplasty by Wullstein and by Zollner describe the methods that they have

developed for reconstruction of the diseased conduction apparatus to improve hearing. A chapter on the use of prosthesis in middle ear deafness is contributed by Dr. Kobrak.

The progress made in the treatment of conduction deafness during the last twenty years becomes clearly evident on reading this book. The progress in our understanding of the function of the conduction apparatus is also made apparent.

The book reflects the shift in emphasis in ear surgery during the last twenty years from that of restoration of general health and prevention of serious complications at the sacrifice of the conducting apparatus to that of preservation or improving function with preservation or reconstruction of all functionally important elements for hearing while at the same time correcting the ear disease.

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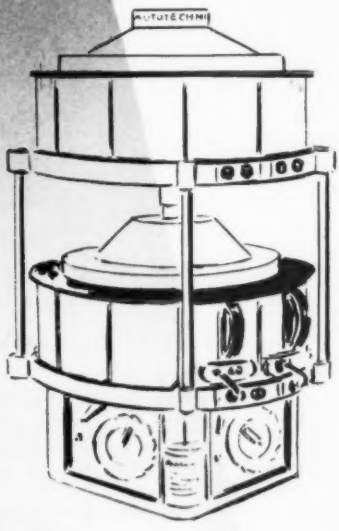

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